Evaluation of the Possible Protective Effect of Alpha Lipoic Acid on Testicular Toxicity Induced by Polychlorinated Biphenyl in Adult Albino Rats: A Histological Study

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Abstract

Introduction: Polychlorinated biphenyl (PCB) is considered one of the environmental pollutants. It is used as hydraulic coils in vacuum pumps, pesticides transformers, heat-exchange systems, capacitors and as additives in adhesive inks, paints, plastics, copying paper and sealants. Alpha lipoic acid (ALA) is an antioxidant substance normally present in mitochondria as a coenzyme. Aim of the Work: To evaluate the protective effect of ALA on PCB induced testicular toxicity. Materials and Methods: Twenty five adult male albino rats were used in this study. They were divided into four groups, a control group included 10 rats, group II rats received alpha lipoic acid 25mg/Kg/day orally for 30 days, group III rats received PCB 5mg/Kg/day orally for 30 days and group IV rats received both PCB and alpha lipoic acid at the same previous dose for 30 days. At the appropriate time, the specimens were taken and prepared for light and electron microscope study. Results: LM examination revealed structural alterations in group III in the form of wide spaces between seminiferous tubules that contain homogeneous acidophilic substance, partial or complete detachment of the tubules from the basement membrane and total distorted irregular shaped tubules. Also dilated congested blood vessels were seen. EM examination of this group revealed Sertoli cells with cytoplasmic vacuolation and dilated rER. The basement membrane appeared as thick and irregular line under Sertoli and spermatogenic cells and it was interrupted in some points. Primary spermatocyte appeared shrunken while others revealed vacuoles in the cytoplasm and perinuclear dilatation. Leydig cells showed irregular vacuoles and swollen destroyed mitochondria. Amelioration of the previous histological changes could be detected in group IV. Conclusion: It could be concluded that alpha-lipoic acid has a protective effect against PCB induced testicular toxicity.

Keywords: Alpha lipoic acid, polychlorinated biphenyl, rats, testis

INTRODUCTION

Polychlorinated biphenyl (PCB) is considered as one of the environmental pollutants. It is used as hydraulic coils in vacuum pumps, pesticide transformers, heat-exchange systems, and capacitors and as additives in adhesive inks, paints, plastics, copying paper, and sealants. PCBs have a chemical formula consisting of C12H10–nCln. Aroclor 1254 is a commercial form of PCBs, and it contains about 54% chlorine in its structure.[1-3]

PCBs spread through water, air, and soil. The oral route through ingestion is the main way of exposure among people. PCBs accumulate more extensively in fatty tissues due to their great lipophility. Previous studies suggested that PCB (Aroclor 1254) has many dangerous effects on the male reproductive system in humans and animals.[3,4]

The cycle of epithelium lining the seminiferous tubule is defined as the series of changes occurring in a certain area of the epithelium between two successive appearances of the same cell association. It takes about 12 days in rats and the spermatogonium takes about four cycles to complete its differentiation. There is a single layer of flat polygonal cells forming a continuous sheet around the seminiferous...
tubule. Those cells are the myoid cells, which possess some ultrastructural features of the smooth muscle cells like contractility, so they are responsible for contraction of the seminiferous tubules. Also these cells contain surface receptors for testosterone and have an important role in the blood–testis barrier.[5,6]

Alpha lipoic acid (ALA) is an antioxidant substance present in the mitochondria as a coenzyme. (lipoic acid;1,2,-dithiolane-3-pentanoic acid). It is considered as a very important cofactor for the function of many enzymes that are involved in energy production and metabolism of fatty acids.[7]

Several authors have studied the potential effects of exogenous LA as a therapeutic agent and can be used for the prevention and treatment of free radical-release-related diseases. It was also proved that pretreatment by ALA had a protective effect against the toxic effects induced by Adriamycin and cyclophosphamide on rat testis. Hence, it is important to study this possible protective effect of ALA on PCB-induced testicular toxicity.[8-11]

**Materials and Methods**

In this study, we used 25 adult male albino rats weighing about 150–200 g. Those rats were kept in clean cages with good ventilation and free access to a standard laboratory diet and water. The experiment followed the standard guidelines of the Local Ethics Committee of Tanta University, Faculty of Medicine, Egypt. The animals were randomly divided into the following four groups.

**Group I (Control group)**

It included ten rats; they were equally divided into two subgroups:

- Subgroup Ia: Five rats received no treatment for 30 days
- Subgroup Ib: Five rats received 2 ml/kg/day of commercial corn oil orally for 30 days.

**Group II**

It included five rats; they were given ALA (ALA, Sigma T5625) (25 mg/kg of body weight [bw]/day by an orogastric tube) dissolved in 2 ml/kg of body weight of corn oil for 30 days.[12]

**Group III**

It included five rats; they were given 5 mg/kg of body weight PCB Aroclor 1254 (Sigma 48,586) dissolved in 2 ml/kg of body weight of commercial corn oil daily by an orogastric tube for 30 days.[12]

**Group IV**

It included five rats; they received Aroclor followed after 1 h by ALA for 30 days at the same previous mentioned doses. At the end of the experiment, animals were weighed and then anesthetized by intraperitoneal pentobarbital injection at a dose of 40 mg/kg.[13] Blood samples were collected directly from the heart of the animals to measure the level of testosterone hormone. Serum was obtained after centrifugation and stored at −20°C. The testes of all animals were dissected out and processed for light and electron microscopy.

**For light microscopic examination**

Specimens were immersed in Bouin’s solution and then washed, dehydrated, and finally embedded in paraffin. Sections of 5 µm thickness were stained with hematoxylin and eosin (H and E).[14]

**For transmission electron microscopic examination**

Testis specimens were cut into small pieces and fixed in 4% phosphate-buffered glutaraldehyde (0.1 M, pH 7.3) for 1 h and then ultrathin sections (80–90 nm) were prepared for examination by a JEOL-JEM-100 transmission electron microscope (Tokyo, Japan) at the electron microscopic unit of Tanta Faculty of Medicine.[15]

**Statistical analysis**

The collected data were analyzed using one-way analysis of variance and by Tukey’s test for comparison between the four groups using Statistical Package for the Social Sciences (SPSS) software (version 11.5; SPSS Inc., Chicago, Illinois, USA). All values obtained were expressed as mean ± standard deviation. Differences between the statistical data were considered statistically significant if $P < 0.05$ and highly significant if $P < 0.001$. [16]

**Results**

In the present study, no deaths were reported throughout the whole experimental period.

**Body weight**

The weight of the two subgroups of the control group and Group II was nearly similar. The mean weight of Group III (Aroclor-treated group) was 156.72 ± 4.63, and it was significantly less than that of the control group (176.01±4.82), and a nonsignificant difference was reported between Group IV (Aroclor and ALA) (161.11 ± 4.25) and the control group at the end of the experiment [Table 1].

**Hormonal assay**

The serum testosterone level of the two subgroups of the control group and Group II was nearly similar. Serum testosterone level in Group III (Aroclor-treated group) showed a highly significant decrease (24.2 ± 2.61) compared to the control group (39.91 ± 2.94), whereas a nonsignificant difference was

| Table 1: Statistical analysis of mean body weight and serum testosterone level |
|-----------------------------|------------------------|------------------------|------------------------|------------------------|
| Parameters                  | Group I                | Group II               | Group III               | Group IV               |
| Mean body weight gain (gm)  | 151.25±5.01            | 176.01±4.82*           | 156.72±4.63             | 161.11±4.25            |
| Serum testosterone (ng/dl)  | 39.91±2.94             | 41.65±4.22             | 24.2±2.61**             | 37.63 ± 3.05           |

Data was expressed as mean±SD. *Indicates significant, **Indicates highly significant. SD: Standard deviation
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Histological results

**Light microscopic examination**

Examination of H and E-stained sections of testis of both control and ALA (Group II) groups revealed no difference between the two groups. They showed that the testis is formed of seminiferous tubules, bounded by basement membrane (BM) and in between the tubules there is interstitial tissue with many Leydig cells. The seminiferous tubules were lined by Sertoli cells and germ cells in different stages of growth, which arranged in layers. Spermatogonia with their characteristic dark rounded nuclei were present on the BM, next to it primary spermatocytes, and early spermatids were found near the lumen. Spermatozoa are present in the lumen of the seminiferous tubules [Figure 1a and b].

Sections from Group III PCB (Aroclor treated) revealed the seminiferous tubules separated by wide spaces that contain homogeneous acidophilic substance and few interstitial cells in between the tubules. Partial or complete detachment of the tubules from the BM was detected [Figure 2a and b].

Totally distorted irregular shaped tubules were observed with detachment of the seminiferous epithelium from the underlying BM leaving one layer of cells on the BM. Other tubules showed wide spaces in between the seminiferous epithelium. Also dilated congested blood vessels were seen and reduced interstitial cells which were replaced by homogenous acidophilic substances [Figure 3a and b].

The most prominent findings in group III were total loss of the cytoplasm mainly in the basal cells with very dark small pyknotic nuclei while, others showing clear cytoplasm around the nucleus. Many vacuoles were detected on the BM separating it from the rest of the seminiferous epithelium. Small acidophilic bodies were found mainly in the basal part of the tubules. Lumen of most tubules was empty or contains few numbers of sperms. Most of these sperms were deeply embedded in between the basal cells of the tubules. The seminiferous epithelium was lost and most of the tubules were lined by two layers of cells, also the interstitial cells appeared with vacuolated cytoplasm [Figures 4, 5 and 6a and b].

Specimens from Group IV revealed improvement in the histological picture in the form of decreased spaces between the seminiferous tubules that contain intact interstitial tissue with Leydig cells. The seminiferous epithelium nearly restored its normal height with spermatogonia at different stages of development [Figure 7a and b].

**Transmission electron microscopy**

Examination of ultrathin sections obtained from the control group revealed the characteristic ultrastructure appearance of the testis. The wall of the seminiferous tubules formed of Sertoli cells and spermatogenic cells. Sertoli cells appeared resting on BM with large euchromatic nucleus. Its cytoplasm showed rough endoplasmic reticulum (rER) and mitochondria. Beside Sertoli cells, the spermatogonia appeared as dome shaped cells with oval nuclei. Next to Sertoli cell, the primary spermatocytes showed large rounded nuclei with dispersed heterochromatin [Figure 8a].

Spermatids were small cells that showed different stages of development. They contained euchromatic nuclei and acrosomal cap. Also, they had specific vacuolated mitochondria peripherally located, and many rER [Figure 8b].

Leydig cell was seen in the interstitial tissue. It showed large slightly rounded nucleus with peripheral heterochromatin and prominent nucleolus. Its cytoplasm contained well developed...
smooth endoplasmic reticulum (sER), variable shaped and sized mitochondria and lipid droplet [Figure 8c].

Examination of ultrathin sections from Group III PCB (Aroclor treated group), revealed different changes in Sertoli cells in the form of dilatation of rER and cytoplasmic vacuolation [Figure 9a and b]. Some of these vacuoles separate Sertoli cell from the surrounding cells and detach it from BM [Figure 10a and b]. Other Sertoli cells showed area of cytoplasmic loss, rarified cytoplasm and many lipid droplets [Figure 11a]. The BM appeared as thick and irregular line under Sertoli and spermatogenic cells and appeared interrupted in other points [Figure 9a and b]. A primary spermatocyte appeared shrunken and separated by wide spaces from other surrounding cells. Other primary spermatocyte revealed cytoplasmic vacuoles and perinuclear dilatation [Figures 9a,b and 10b].

Spermatids were the most affected cells and revealed variable lesions in the form of heterogeneous electron dense body in the cytoplasm and they showed also large acrosomal cap with irregular nucleus and disturbance in the nuclear membrane [Figure 11a]. Multiple irregular shaped vacuoles were detected in the cytoplasm of the spermatids [Figure 11b]. Other spermatids which located near the lumen, showed nuclear deformities in the form of a mass of condensed chromatin and absence of acrosomal cap. Multiple irregular cytoplasmic vacuoles of variable sizes and shapes, areas of rarified cytoplasm, dilated mitochondria and rER were observed [Figure 12a and b].
Figure 7: A photomicrograph from Group IV showing (a) two seminiferous tubules lined with multiple layers of spermatogenic cells, lumen contains mature sperms (s), intact interstitial tissue with Leydig cells (thick arrow). (b) Part of a seminiferous tubule contains spermatagonia (bifid arrow), Sertoli cells (arrow), primary spermatocytes (curved arrow) and early spermatids (wavy arrow) (H and E a ×400, b ×1000)

Figure 8: Control group showing (a) Sertoli (Se) cell and spermatogonia (Sg). Sertoli cell has large euchromatic nucleus (N), Rough endoplasmic reticulum (R) and mitochondria (m). Spermatogonia with oval nucleus (N) a primary spermatocyte (Ps) with rounded large nucleus (N). (b) Early spermatids contain euchromatic nucleus (N) and acrosomal cap (wavy arrow). Characteristic vacuolated mitochondria (m) peripherally located, and many rough endoplasmic reticulum (R). (c) Leydig cell with rounded nucleus (N) peripheral heterochromatin and prominent nucleolus. Smooth endoplasmic reticulum (arrow), mitochondria (m) and a lipid droplet (d) (TEM; ×1500 and ×3000 and ×2500)

Figure 9: EM from Group III showing (a) Sertoli cell with its characteristic nucleus (N) resting on irregular, interrupted thick basement membrane (arrow head). The cytoplasm showing vacuoles (V) dilated rough endoplasmic reticulum (R) and normal mitochondria (m). Notice presence of part of myoid cell under the basement membrane (MY). (b) Parts of 2 Sertoli cells with their characteristic nuclei (N) and spermatogonium (SG) in between. These cells resting on irregular corrugated basement membrane (arrow head) and Sertoli cell shows cytoplasmic vacuoles (V). Also, an irregular shrunken primary spermatocyte (P) separated by large irregular vacuoles (V) from the surrounding structures (TEM; ×3000 and ×1500)

Figure 10: EM from Group III showing (a) Sertoli cell with large dark nucleus (N), area of cytoplasmic loss (star) and vacuoles (V) separate it from the basement membrane and the surrounding cells. Increased intercellular space between the primary spermatocyte (P) and the cells around it (arrow). A part of a sperm deeply impeded between the cells (bifid arrow). Notice thick basement membrane (arrow head). (b) Part of Sertoli cell shows large cytoplasmic vacuoles (V) and irregular detached basement membrane (arrow head). Also there is a part of primary spermatocyte (P) with cytoplasmic vacuoles (V) and perinuclear dilatation (curved arrow) (TEM; a ×1500 and b ×3000)

Interstitial tissue was affected and showed changes in Leydig cells with excessive collagen fibers deposition around the fibroblast cells. Leydig cells revealed irregular cytoplasmic vacuoles and absence of lipid droplets while, other cells had few lipid droplets. Leydig cells also revealed dilated sER, swollen destroyed mitochondria and totally destroyed cells with loss of most cellular content were detected. The nuclei of Leydig cells were normal with its characteristic shape [Figure 13a and b].

Results obtained from Group VI (protective group) clarified manifestation of improvement in general however few lesions were still present as few cytoplasmic vacuoles of Sertoli cells [Figure 14a]. Most of spermatids appeared normal and...
restored normal contact between the cells [Figure 14b]. Few spermatids at acrosomal stage showed irregular shaped nucleus and secondary lysosomes in their cytoplasm, also there were wide intercellular spaces present between them [Figure 15a]. As regard Leydig cells depicted normal shaped mitochondria and variable sized lipid droplets, but multiple dilated sER and some vacuoles were detected [Figure 15b].

**DISCUSSION**

Testosterone hormone has a crucial role in the initiation and maintenance of spermatogenesis with quantitative and qualitative regulation of spermiogenesis process, so reduction of testosterone levels causes a decrease in the number of spermatogenic cells mainly due to altered function of Sertoli cells that deprives the developing spermatogenic cells from the necessary factors rather than to a direct effect on the germ cells.[17,18]

In the present study, serum testosterone was significantly decreased in the PCB treated group. This was in consistent with Murugesan et al.[19] and Han et al.[20] Kumar et al.[21] who explained this steroidogenesis suppression by suppression of luteinizing hormone (LH) secretion, so implicating the pituitary–gonadal axis as a target for this endocrine disruption.[22,23]

Steroidogenesis requires a special protein called steroidogenic acute regulatory (STAR) protein, which is important for cholesterol transport to the mitochondrial membrane to enhance its usage by the steroidogenic enzymes, Kumar et al.[21] reported a decreased formation and transcription of STAR in the testis resulting in decreased testosterone level.
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Widening of the intertubular spaces was detected in the present study which was also observed by Abdellatief et al.\cite{27} and El-Sherif and El-Mehi\cite{29} who attributed this to the deposition of a homogenous acidophilic Periodic-acid Schiff positive material or a hyaline material. This finding may be also due to oozing of excess lymphatic exudate from the degenerated lymphatic vessels as well as increase in vascular permeability that may result from deposition of ROS and free radicals.\cite{29}

The wide intercellular spaces between spermatogenic cells detected in our study, were also detected by Mohamed et al.\cite{30} who attributed this to exposure of spermatogenic cells to ROS causing disturbances of the blood–testis barrier, allowing passage of toxic agents between the cells and the widened of intercellular spaces.

Cytoplasmic vacuolation of most spermatogenic cells and Sertoli cells observed in our work, are most probably derived from dilatation and vesiculation of the endoplasmic reticulum and mitochondrial swelling, while the larger vacuoles are often due to phagocytic vacuoles remaining after the digestion of necrotic germ cells. Also, the vacuolated cytoplasm could be due to lipid peroxidation which leads to damage to the cell membrane, or it could be a result of disturbed plasma membrane permeability that losses the ability to maintain ionic and fluid homeostasis.\cite{30,31}

In the present work, marked depletion of the spermatogenic population, atrophy of some tubules, focal disorganization and shrunken seminiferous tubules were detected. These results coincided with Cajú et al.\cite{32} and Abdellatief et al.\cite{27} who explained these findings by disturbance in the endocrine functions through disturbed LH, estradiol, somatostatin, prolactin and gonadotrophin releasing hormone levels.

In this research, small pyknotic nuclei, clear cytoplasm around the nucleus and cytoplasmic vacuoles were found in cells lining the seminiferous tubules mainly in the basal, these findings could be explained as a feature of apoptosis which increased due to profuse production of ROS.\cite{33}

In our research, the cytoplasm of Leydig cells showed many vacuoles and dilated sER and these findings coincide with Ghoneim et al.,\cite{34} Ahmed and Kurkar\cite{35} and Abdel-Zaher et al.\cite{36} who reported malformed Leydig cells with reduction in their number and attributed this to the progressive increase in testicular levels of nitric oxide and to oxidative stress.\cite{17}

Moreover, the histological alterations in Sertoli and Leydig cells in this context has been attributed to the decreased testosterone levels resulting in histological changes in these (androgen target) cells.\cite{38,39}

In the present work, collagen fibers deposition around the fibroblast cells was detected in the interstitial tissue. Some authors attributed collagen deposition in the interstitial space of the testis to the production of reactive oxygen species (ROS), increase lipid peroxidation and decrease the activity of antioxidant enzymes such like glutathione peroxidase in the epididymal sperms and the mitochondria of the testicular cells.\cite{19,26}

These previous data explained our observation why the lumen of most tubules was empty or contains few numbers of sperms.

In our research, there was a significant decrease in the body weight in PCB treated group. This was in agreement with Ateşşahin et al.,\cite{2} Aly et al.,\cite{14} and Murugesan et al.\cite{19} who reported that intraperitoneal administration of PCB significantly decreased the whole body and the testis weight.

In the Aroclor group of this work we reported detachment of the seminiferous epithelium from BM and wide areas of lost seminiferous epithelium with vacuolated cytoplasm, these results coincided with Han et al.\cite{20} and Cai et al.\cite{28} who reported that Aroclor 1254 exposure reduces the weight of testis and epididymis, produces seminiferous tubules degeneration and damages spermatogenesis and decreases serum testosterone level, decreases number of sperms count and affects their motility with increased apoptotic cells in the testis.

One of most prominent finding in this work was deeply embedded sperms in between the basal cells of the tubules and the heterogeneous electron dense bodies observed in the cytoplasm of the spermatids, which most probably were residual bodies. These observations could be due to disrupted skeleton of the Sertoli cell that appears to be one of the first targets of the environmental toxicants. The skeleton of Sertoli cells is based on the polarized microtubules (MT) which is essential for the transport of residual bodies and spermatids across the lumen. So, defects in MT result in failure of spermatid transport leading to the deeply embedded spermatids inside the epithelium and germ cell apoptosis.\cite{25}

Light and ultrastructural changes observed in this work could be explained that PCB exposure may result in the production of reactive oxygen species (ROS), increase lipid peroxidation and decrease the activity of antioxidant enzymes such like glutathione peroxidase in the epididymal sperms and the mitochondria of the testicular cells.\cite{19,26}

Figure 15: EM from group IV showing (a) Spermatids near the lumen, some of them with normal acrosomal cap (arrow) and granule. Others showing irregular shaped nucleus (N) and secondary lysosome (curved arrow) in its cytoplasm. Wide intercellular space present between the cells (star). (b) Two Leydig cells with normal shaped mitochondria (m), multiple dilated smooth endoplasmic reticulum (wavy arrow), some vacuoles (V) and variable sized lipid droplets (d) are seen. Notice the characteristic shape of the nucleus (N) (TEM; ×1500 and ×2500)
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is to fill the wide space in between the tubules and this space resulted from germ cell loss and explained the presence of collagen fibers is due to decreased testosterone secretion. Also, collagen deposition might be due to increase its formation by the myoid cells or due to proteolysis rate reduction in the extracellular space.

In the present research, animals treated with both PCB and ALA showed improvement of the previous histological changes occurred in PCB treated group. This goes in line with Gülş and Eren[12] who proved that ALA reduced the effects of oxidative stress in PCB toxicity. Other authors proved that ALA has an extraordinary antioxidant effect that protects the testis against toxic effects. They explained this effect due to the lipophilic nature ALA which can easily cross cell membranes and effectively remove the free radicals.[13]

CONCLUSION

Finally, it is concluded that PCB administration produces different histological harmful effects on the testis of adult albino rats and they can be prevented to some extent by ALA administration.

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Conflicts of interest

There are no conflicts of interest.

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