

Chronic Toxic Effect of Triclosan on Reproductive System of Albino Rats

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Abstract:

Background: Triclosan (TCS) is a widely used antiseptic compound that has been added to personal care products. Triclosan is absorbed through mucous membranes of the oral cavity and gastrointestinal tract after oral exposure and through the skin after dermal exposure. It is suspected to be a potential reproductive toxicant. **Aim:** assessment of the chronic toxicity of triclosan on testes and ovaries of normal adult albino rats through measuring the level of hormones as testosterone, estrogen ,FSH and LH and assessment of histopathological and ultrastructural changes in testes and ovaries . **Methods :** A total of 80 normal adult albino rats (40males and 40 females) were used ,the animals were divided into 8 equal groups (10 rats per each group) as follows: Group-I (Male Control Group),Group-II (Female Control Group),Group-III (Vehicle-Treated, Acacia Gum Treated Male Group),Group-IV (Vehicle-Treated, Acacia Gum Treated Female Group),Group-V (Triclosan orally Treated Male Group),Group-VI (Triclosan orally Treated Female Group), In Group-VII (Triclosan dermally exposed male Group , group VIII(Triclosan dermally exposed Female Group), the groups received doses of triclosan(For oral groups:435 mg/kg body weight, For skin exposed groups: 600 mg/kg body weight) for twelve weeks . **Results:** Decreased levels of measured hormones, Low sperms count and motility, histopathological and ultrastructural changes in testes and ovaries in oral and dermal exposed groups. **Conclusion:** Triclosan caused low levels of sex hormones as testosterone, estrogen, FSH and LH, It affected sperms count and motility and It caused histopathological and ultrastructural changes in testes and ovaries.

Key words: Triclosan, testes, ovaries

List of Abbreviations:

ATP: Adenosine Triphosphate

cAMP: Cyclic Adenosine Monophosphate

EDC: endocrine-disrupting chemical

ENR: enoyl-acyl carrier protein reductase enzyme

ER : estrogen receptors

EST: estrogen sulfotransferase

FabI: fatty acid biosynthesis I

FSH: Follicle-stimulating hormone

HPG: hypothalamic–pituitary–gonadal

LH: Luteinizing hormone

NAD⁺: nicotinamide adenine dinucleotide

OS: oxidative stress

ROS: reactive oxygen species

STs: seminiferous tubules

TCS: Triclosan

Introduction

Triclosan (5-chloro-2-[2, 4-dichlorophenoxy] phenol) has an anti-microbial broad spectrum activity against both gram-negative and gram-positive bacteria (1).

Triclosan (TCS) is a commonly used compound applied to personal care items such as hand soap, shampoos, deodorants, toothpastes, mouthwash, cosmetics, cleaning and pesticides, as well as consumer goods such as kitchen utensils, toys and impregnated in different materials such as athletic apparel and food packaging (2).

TCS is commonly used in surgical scrubs and hand washings with a total contact period of around two minutes. It is also used in the coatings for some surgical sutures and to decolonize the skin of methicillin-resistant *Staphylococcus aureus* (3).

At the lower concentrations seen in commercial products, TCS acts as a bacteriostatic agent primarily by inhibiting bacterial cell membrane synthesis of fatty acids (4).

Fatty acids are necessary for cell membrane construction and reproduction. Triclosan binds to the enzyme bacterial enoyl-acyl carrier protein reductase (ENR), which is encoded, by the gene FabI (fatty acid biosynthesis I) and increases the affinity of the enzyme to the adenine dinucleotide (NAD⁺) nicotinamide. This results in the formation of a stable, ternary ENR-NAD⁺-triclosan complex that cannot participate in fatty acid synthesis (5).

Staff can be exposed to dermal touch and inhalation when processing TCS. Based on a National Occupational Exposure Survey conducted between 1981 and 1983, the United States National Institute for Occupational Safety and Health reported that 188,670 workers in 16 different industries in the United States are potentially exposed to TCS (6).

The persistent discharge, bioaccumulation and degradation resistance into the sewage

system are leading causes of significant environmental and public health hazards. Consequently, significant levels of TCS are widely detected in different environmental compartments (such as surface water, sediment, soil, and aquatic species) and in human body fluids (7).

Triclosan reaches the systemic circulation through absorption by various routes such as oral cavity mucous membranes; gastrointestinal tract after oral exposure and skin after dermal exposure (8).

Because of its high lipid solubility, it is readily absorbed from the gastrointestinal tract with peak plasma concentration within 1 to 3 hours with subsequent fecal, urinary and breast milk excretion (9).

Age and gender also have a significant influence on serum TCS concentrations, which are higher in males than females, and the highest in the age group between males and females aged 31-45 years (10).

Many everyday hygiene products that contain TCS are routinely exposed to humans of all ages; however, the toxicological and biological effects of TCS in the human body following long-term and low-concentration exposure are far from well understood (11).

In human safety studies that exposure to TCS from many consumer products (e.g. toothpaste, mouth rinses, or aqueous slurry)

would not be expected to cause adverse health effects in humans (12).

Despite this, TCS was shown to be cytotoxic to certain forms of breast cancer cells when stored in vitro at various concentrations (13).

TCS also, inhibited adipocyte differentiation only at high concentrations when grown with human mesenchymal stem cells. In addition, TCS arrested human endometrial stromal cells in the cell cycle phase enhancing cell migration, increased gene expression and protein levels of insulin growth factor binding protein 1 and prolactin, and increased the effect of progesterone, so it is strongly suggested that TCS could alter human endometrial physiology, thus affecting fertility and pregnancy outcomes (14).

Several experimental studies have shown that TCS can induce hepatic tumors in mice that could be attributed to its peroxisome proliferator-activated receptor activation α and neurodegenerative changes in rat brain and dam development through mechanisms involving activation of reactive oxygen species and initiation of apoptosis (15).

TCS could also be a low-dose endocrine disruptor and disturb thyroid hormone homeostasis by inhibiting iodide uptake, and inhibiting thyroid peroxidase activity. TCS induced inhibition of circulating steroid hormone production has been reported to be associated with altered

expression of hormone metabolism enzyme genes in the placenta. This disruption of the hormone will eventually affect the development and growth of the fetus (16).

Triclosan inhibits oestrogen sulfonation by interacting with sulfotransferases, elevating TCS concentrations of oestrogen is potentially relevant to the anti-reproductive and carcinogenic actions of excessive oestrogen activity, particularly breast cancer (17).

It is also suspected of being a potential reproductive toxicant for males due to its anti-androgenic activity. This chemical has been shown to reduce the weights of testes and sex accessory organs, sperm density and testosterone production in Leydig cells and to disturb the function of major steroid enzymes (18).

In men, an association has been observed between environmental exposure to TCS and poor semen quality parameters, indicating that TCS may affect human sperm production and normal morphology. The TCS action threshold on semen can be quite low and most men are sensitive to TCS at that level (19).

The US FDA banned the sale of 'consumer antiseptic washes' containing triclosan (20).

Due to Triclosan's worldwide high volume of dermal exposure and its significant level of exposure from various products in all age groups for life-time duration, the Food

and Drug Administration (FDA) issued a rule stating that over-the-counter consumer antiseptic wash products containing many potentially harmful antibacterial active ingredients — including triclosan— can no longer be marketed to consumers. These products include liquid, foam and gel hand soaps, bar soaps, and body washes. It also recommends that triclosan not be used in the home, as it may encourage bacterial resistance to antibiotics (21).

Triclosan is not an essential ingredient in many products. There is no evidence that antibacterial soaps and body washes containing triclosan are more effective than plain soap and water in preventing illness and the spread of certain illnesses (22).

Many manufacturers have started removing this ingredient from their products. It has been proved that triclosan-containing antibacterial soaps neither safe nor effective. Also, triclosan is no more effective than plain soap yet present significant safety concerns for people and the environment. So, the time has come to wash our hands of triclosan and other unnecessary antimicrobial chemicals for good (23).

Aim of the Work

The present work aimed at assessment of the chronic toxicity of triclosan (TCS) on testes and ovaries of normal adult albino

rats of both sexes through biochemical, histological and ultrastructural examination.

Material and Methods

A-Technical Design

Study Design: An experimental controlled case control trial study

Study Setting: Forensic and clinical Toxicology department, Faculty of medicine, Benha University

Time of study: 3 consecutive months from October 2019 to January 2020

Material:

(I)- Characters of Animals:-

Adult Sprague-Dawley albino rats of both sexes were used in this work (mature rats with closed epiphysis, 3-4 months). They were obtained from the animal house of Hellwan's Farm for Experimental Animals.

This work was performed on 80 adult male and female albino rats. According to the Ethics Committee of Scientific Research, Faculty of Medicine, University of Benha, every 10 rats were housed in separate, clean, and were ventilated cage under strict care and hygiene to keep them in normal and healthy conditions. Free access to food and water were allowed. The

animal was anesthetized before taking the samples.

(II)- Husbandry:-

In order to exclude fallacies induced by environmental factors the following environmental conditions:

Suitable climatic conditions in the animal house, suitable type of bedding, low noise and the study was performed in summer in sufficient natural light. Before commencing experimentation, all the animals were subjected to one week period of passive preliminaries in order to adapt themselves to their new environment, to ascertain their physical wellbeing, and to exclude any diseased animals (24).

All animals received the same diet (Wheat, Bread & Milk). The time of drug administration were fixed for all animals at 12p.m.

(III)- Chemical:

Triclosan, (Sigma-Aldrich Company), is a white to off-white crystalline powder readily soluble in alkaline solutions and many organic solvents (25), but only slightly soluble in water (10 mg/L at 20 degree °C) (26). For oral route, the LD₅₀ of triclosan for rats is between 3,700 and 5,000 mg/kg body weight (27), so its average 1/10th LD₅₀ equal 435 mg/kg body

weight. For skin exposure, the LD50 of triclosan for rats is 6000 mg/kg body weight (28) so its average 1/10 LD50 equal 600 mg/kg body weight.

Operational Design:

(IV)- Experimental Design Regimen, Treatment Regimen, and Duration of the Study:-

A total of 80 normal adult albino rats (40 males and 40 females) were used in this study. The animals were divided into 8 equal groups (10 rats per each group) as follows:

- Group-I (Male Control Group).
- Group-II (Female Control Group).
- Group-III (Vehicle-Treated, Acacia Gum Treated Male Group).
- Group-IV (Vehicle-Treated, Acacia Gum Treated Female Group).
- Group-V (Triclosan orally Treated Male Group).
- Group-VI (Triclosan orally Treated Female Group).
- Group-VII (Triclosan applied on skin, male group).
- Group-VIII (Triclosan applied on skin, Female group).

Each fasted rat in the 1st 6 groups was subjected to treatment regimen orally via gastric gavage for three consecutive months as follows:

- In Groups I and II, the rats received distilled water.
- In Groups III and IV, the rats received 5% acacia gum suspension.
- In Groups V and VI, the rats were treated with 435 mg/kg body weight suspension of triclosan.

Before each administration, fresh suspensions of 5% acacia gum and triclosan was prepared to obtain the necessary concentrations in 1 ml. All the rats were weighed before administration of their corresponding treatment regimen and the doses were calculated according to their daily weights.

- In group VII and VIII, 600 mg/kg (1/10 LD50 for skin exposure) of triclosan in ethanol: olive oil was applied to the skin of the rats (female, male rats) (28)

(V)- Route of Administration:-

Oral route was applied to 1st 6 groups, skin route to the last group.

(VI)- Observation of Any Abnormalities

Close observation of all animals throughout the experimental periods may reveal several clues to the mechanism(s) by which the toxicant is eliciting its effect or toxic manifestation that might occur as a result of chemical administration. The general appearance of the animals in all groups will be noted and compared together (29).

The following parameters were monitored during the ongoing chronic toxicity studies:-

1. Behavioral changes (sedation and irritability).
2. Musculoskeletal or locomotor activity.
3. Changes in body weight.
4. Skin irritation.

(VII) Collection of samples

Twenty four hours after the end of the experimental period, the animals were anesthetized by diethyl ether inhalation, weighted, then subjected to thoracolaparotomy incision to obtain blood samples from the heart directly by needle aspiration and remove required organs and then the animals were sacrificed by decapitation. The deceased animals were eliminated by incineration in Benha university hospital incinerator.

A- Seminal analysis:

The epididymis was dissected out then process for spermatozoa count or concentration, sperm motility, and sperm morphology by using haemocytometer (30).

B- Biochemical tests:

Blood samples were collected in red-topped tubes, left to coagulate, centrifuged and the supernatant sera were removed, collected in eppendorf tubes, and immediately stored at -20°C till been assayed.

Biochemical analysis of hormones such as luteinizing hormone (LH), follicle stimulating hormone (FSH), estrogen, and testosterone were assayed according to the methods described inside the manufacturer's instructions of the supplied commercial diagnostic kits.

C- Histopathological preparations:

One part from testes and ovaries was fixed in Bouin's solution, subsequently dehydrated in ascending grades of alcohol, cleaned by xylene, and embedded in paraffin wax, then sectioned at 4 µm thicknesses by a microtome (31). Tissue sections were stained with Hematoxylin and Eosin (Hx and E) and then mounted slides were examined and photographed using light microscope.

D- Ultrastructural preparations:

Thin sections from collected tissues were prepared according to the following method: Briefly, 1-3 mm segment of tissues were fixed in fresh 3% glutaraldehyde-formaldehyde at 4 °C for 18–24 h. The specimens were washing in phosphate buffer (pH 7.4) and post-fixed in isotonic 1% osmium tetroxide for 1 h at 4 °C and then processed. Semithin sections (1 µm) were stained with toluidine blue and examined under the light microscope to confirm the presence of tissue changes. Ultrathin sections (70-80 nm) were obtained from altered tissue sites, mounted

on copper grids, and stained with uranyl acetate and lead citrate. Finally, each grid was examined and photographed using transmission electron microscope (32).

(VIII)- Statistical Analysis:

The collected data were tabulated and analyzed using SPSS version 16 software (SPSS Inc, Chicago, ILL Company). Quantitative data were expressed as mean \pm standard deviation (\pm SD) and range. The accepted level of significance in this work was stated at 0.05 ($P < 0.05$ was considered significant) (11).

(IX)- Ethical Considerations:

Institutional review board approval of protocol of this study was obtained.

RESULTS

A) Biochemical study:

In the present work, triclosan orally treated rats at dose 435 mg/kg/day for 12 weeks showed a significant decrease in the mean value of testosterone, a significant decrease in the mean value of estradiol as compared to control and vehicle group.

In comparing mean value of skin treated testosterone and control and vehicle groups. Testosterone is lower in treated than control and vehicle groups respectively and mean value of, estrogen is lower in skin treated female group than control and vehicle groups respectively.

The present study showed a non-significant ($p > 0.05$) decrease in mean values of follicular stimulating hormone (FSH) levels in male triclosan orally and dermally treated group in comparison to control and vehicle groups, Also there were a non-significant decrease in mean values of luteinizing hormone (LH) levels in male triclosan orally and dermally treated group in comparison to control and vehicle groups. The current study showed a non-significant ($p > 0.05$) decrease in mean values of FSH and LH levels in triclosan orally female treated group in comparison to control and vehicle groups.

Histological results (H&E stain)

Orally treated triclosan male group:

Examination of H&E-stained testis parts in this community showed marked morphological changes for most of the seminiferous tubules. There was a significant reduction in the spermatogenic cell mass with a consequent thinning out of the layers of lining cells that exhibited deeply stained nuclei. Individual cell loss was demonstrated through empty spaces between sperm cells.

Furthermore, many seminiferous tubules have revealed cytoplasmic vacuolation of the lining cells. Furthermore, the seminiferous tubules attained relatively broad lumina in the lumen with little sperm. Exfoliated germ cells also frequently found in the tubular lumen.

Between the seminiferous tubules, the interstitial Leydig cells were seen, many of which had dark nuclei.

In triclosan dermally treated male group; Parts of the testicular system showed seminiferous tubules of varying sizes & shapes with degenerated germ layers. The spermocytes themselves had degenerated. Additionally, vacuolations between seminiferous tubules were seen in the stroma.

In triclosan orally -treated female group; sections of the ovaries had degeneration in the follicular cells with stroma vacuolations.

While a triclosan dermally –treated female rat, it showed degeneration in follicular cells with stroma vacuolations but to a lesser extent.

Ultrastructure results:

Triclosan dermally treated male group

Triclosan treated male rat showing abnormal sperm cells with irregular nuclei and greater quantities of heterchromatin in addition to intercellular space, abnormal Sertoli cell , abnormal seminal tubules with apoptotic cell and vacuolations, abnormal primary spermatocyte and irregular nucleus, swollen mitochondria and irregular nucleus vacuolations, condensed chromatin and vacuolations, in addition to lysosomal decrease.

Triclosan orally treated male group:

Ultrastructural examination of rat testis showed evident changes in seminiferous tubules, both degenerative and microvacuolar. The Sertoli cells displayed substantial cytoplasmic vacuolation with endoplasmic reticulum dilatation. The cells also showed degenerated mitochondria with disrupted cristae, and relatively increased lysosomal content; some cells have rarified cytoplasm areas.

All spermatogenic cell types have been affected, too.

Some spermatogonia displayed large cytoplasmic vacuolation, while others had small dense nuclei with clumped chromatin and dense cytoplasmic material. Primary spermatocytes Indicated irregular nuclei with cytoplasmic vacuolation. As for the spermatids, they showed variable-size nuclear deformities and multiple cytoplasmic vacuoles as well as localized areas of cytoplasmic depletion. Additionally, many deformed sperms with abnormal head defects and swollen degenerated mitochondria were frequently found.

Triclosan dermally treated group female (ultrastructure)

Besides the vacuolar degeneration, atretic follicles and corpus luteum hyperplasia can be observed. There is follicular vacuolation and degeneration, with nucleus rarefaction. Triclosan treated rat also displayed

follicular degeneration with apoptotic nucleus and vacuolations (follicular atresia), abnormal corpus luteum with irregular nucleus and large quantities of lipid droplets.

Semen Analysis:

Triclosan decrease all normal features of semen analysis to a greater extent in orally treated group and to a lesser degree in dermally treated group.

Discussion

In the present study, as regard hormonal assay; there was a significant decrease in serum testosterone levels, and a non-significant decrease in serum follicular stimulating hormone (FSH) & luteinizing hormone (LH) levels in triclosan treated (orally and skin) male groups as compared to control male groups.

By evaluation the effect of TCS and its withdrawal on the cauda epididymis of adult albino rat using different histological and biochemical techniques (33). The findings were in a harmony with our results.

Also by assessment of the genotoxic and antiandrogenic potential effects of TCS represented by histopathological and testicular hormonal evaluations (34), there was a significant reduction in serum level of testosterone in male rats treated with triclosan as our results.

By studying the effects of administration of triclosan on testis, a significant decrease in serum testosterone level was detected (35).

TCS as an endocrine-disrupting chemical (EDC) in male rats has the potential to affect the pituitary-gonadal pathway at various levels because of its action at various steps of steroidogenesis: including reduction of LH and cholesterol production; depressed StAR protein expression and finally down-regulation of several key steroidogenic enzymes. That in turn impairs the androgen production .Also oxidative stress, that may be a mechanism way for TCS as an antiandrogenic chemical (36).

It has been speculated that TCS suppresses steroidogenesis as a result of an effect on luteinizing hormone (LH) secretion, thereby implicating the pituitary–gonadal axis as a target for endocrine disruption. It is well known that when LH binds to its receptor in a Leydig cell it results in activation of various agents of steroidogenic cascade, causing an increased production of testosterone (37).

The probable mode of action of TCS as an antiandrogenic compound through treating male albino rats with three doses of triclosan for a period of 60 days followed by the analysis of various biochemical parameters, confirmed the antiandrogenic

activity of TCS in Leydig cells through disruption of the entire cAMP-dependent steroidogenic pathway. Moreover, TCS has been speculated to decrease serum cholesterol level, which, in turn, results in down-regulation of steroidogenesis (38).

Triclosan structural similarity to non-steroidal estrogens, suggests that TCS may affect sex hormones' homeostasis. Although TCS binds to steroid receptors, the consequences of this interaction remain unclear (39).

TCS also, displaced estradiol from estrogen receptors (ER) of MCF7 human breast cancer cells and from recombinant human ER α /ER β which suggest anti-androgenic and anti-estrogenic effects, respectively (40).

By investigation of the potential effect of prenatal TCS exposure on fetal reproductive hormones in cord blood and its potential mechanism in relation to placental steroidogenic enzymes, there is a potential impact of prenatal TCS exposure on reproductive hormones in cord blood mediated by steroidogenic enzymes, and male infants were more vulnerable (41).

Triclosan decrease cAMP production by lowering activity of adenylyl cyclase enzyme, leading to low testosterone biosynthesis (42).

Regarding semen analysis in orally treated group, The results of the present study showed changes in sperm count, motility and viability which were in agreement with other studies who aimed to evaluate the distribution status of TCS in male reproductive organs of rats, and seek the correlation with the TCS-induced sperm toxicity or reproductive organ damage through administration of TCS to rats at a dose of 50 mg/kg (5,18).

However, on dermal treated group, the results of the present study showed non-significant changes in sperm count, motility and viability, same results were found by evaluation the toxicity of triclosan administered dermally to mice for 13 weeks (6).

Dermal studies conducted in both animals and humans indicated dermal absorption of triclosan to be less than that observed following oral administration (43), these results are in the same line with our results.

The decrease in number of sperm production can be due decreased testosterone production in TCS treated rat, which is important for sperm production (44).

Furthermore, low sperm count was recorded in the testis of treated animals as compared with controls, probably because

of reduced testicular spermatogenesis induced by TCS (38).

ATP synthesis by mitochondrial oxidative phosphorylation was dysfunctionalized by triclosan which acts as uncoupler (42).

The current study illustrated that there was a significant decrease in serum estrogen levels and a non-significant decrease in serum FSH & LH levels in triclosan orally and skin treated female rats, compared to control female rats. Other studies have shown the same results by studying the effect of triclosan on these hormones (45, 46).

On the other hand, another study of the influence of triclosan (TCS) on estrogenic activity and thyroid function of rats found that estradiol levels, FSH, LH in the TCS-only exposed ones were significantly increased (47).

One possible explanation is that the TCS exposure causes the inhibition of estrogen sulfotransferase (EST) to reduce the estradiol metabolism and clearance (48).

Triclosan can be both estrogenic and anti-estrogenic depending on the dose and other factors (49).

TCS have been documented to increase expression levels of uterine calbindin-D9k (CaBP-9k). CaBP-9k is a well-known

biomarker for endocrine disruptors detection (47).

It is known that TCS is estrogenic disruptors. One of the mechanisms is disruption of the estrogen system via the estrogen-related receptor γ at human exposure levels (50).

There is a firm association linking the changes in female fertility and thyroid disorders. It was reported that hypothyroidism have an undesirable effect on female reproduction (39).

The results of the present study showed histopathological changes in the testicular specimens of the male rats treated with triclosan including dilatation in seminiferous tubules, oedematous stroma with vacuolations and degenerated spermocytes.

The results of the present study were in agreement with other studies of histopathological alteration caused by triclosan on testes of male rats. The tubules revealed considerable reduction in the number of spermatogenic lining cells with the frequent appearance of deeply stained nuclei. Many tubules exhibited cytoplasmic vacuolation of the lining cells along with exfoliated germ cells in the tubular lumen. Ultrastructurally, Sertoli cells evidenced considerable vacuolation of the cytoplasm

with degenerated mitochondria and prominent lysosomal activity **(51,38)**.

Many cells exhibited small dense nuclei and dense vacuolated cytoplasm, whereas others depicted less degenerative changes with wide perinuclear cisternae. Primary spermatocytes and spermatids were also among the affected germ cells. They showed nuclear irregularities, wide perinuclear cisternae, and cytoplasmic vacuolation **(45)**.

TCS treated rats also showed significant lowered testicular weight, number of germ cells, Sertoli cells, Leydig Cells, primary spermatogonia, secondary spermatogonia, and spermatocytes. These observations suggest that TCS have degenerative and retrogressive effects on rat testes **(44)**.

Histologically, most of the STs retained normal appearance and epithelial stratification. Only some tubules revealed vacuolation of germ cells in the basal compartment with deeply stained nuclei with mild ultrastructural alterations of germ cells. These findings suggest that exposure to TCS results in considerable damaging effect on all spermatogenic stages starting from spermatogonia **(52)**.

TCS administration was associated with degenerative changes in the STs as well as in sex accessory organs of adult rats – namely, the cauda epididymis, vas

deferens, and prostate. They attributed such histological alterations to the decrease in the levels of testosterone and androgen receptors in treated rats **(38)**.

TCS caused histopathologic alterations in the testis and reduced plasma LH and testicular testosterone.. These histological alterations were attributed to the decrease in the levels of testosterone and androgen receptors in treated rats **(53)**.

By ultrastructural examination, there were depicted principal cells with irregular nuclei, vacuolated cytoplasm with many lysosomes, dilated Golgi apparatus and rough endoplasmic reticulum. Irregular apical cytoplasmic projections and sparse stereocilia were observed. Clear cells showed some cytoplasmic projections, large apical vacuoles and few lipid droplets. migrating halo cells with vacuolated cytoplasm also were observed **(33)**.

The testosterone hormone plays a crucial role in the initiation, maintenance, and quantitative and qualitative regulation of spermatogenesis and spermiogenesis processes mainly through its stabilizing action on Sertoli cells. It has been postulated by several investigators that deprivation of testosterone affects these processes and causes a decrease in the number of spermatogenic cells. The

underlying mechanisms have been attributed to the altered function of Sertoli cells that deprives the developing spermatogenic cells from the necessary survival factors rather than to a direct effect on the germ cells, so decreased testosterone levels are associated with histological alterations in Sertoli and Leydig (androgen target) cells (54).

In addition, TCS may have direct effects on mitochondria, impairing its function through an uncoupler effect and disrupting mitochondrial membrane fluidity. Besides, it directly affects ATP, causing a significant inhibition of the enzyme activity, considering the results obtained with disrupted mitochondria (55).

Although the decrease in testosterone production is an important factor implicated in TCS-induced testicular injury, oxidative stress (OS) has been recently suggested as another mechanism responsible for the cytotoxic effect of TCS. TCS at sublethal concentrations induces OS, which decreases the cellular thiol content with a consequent increase in intracellular zinc concentration by its release from intracellular stores in rat cells. It has been postulated that zinc impairs the antioxidative system through NADPH-dependent mechanisms and promotes the intracellular production of reactive oxygen species (ROS). Thus, TCS-induced

disturbance of cellular zinc homeostasis may induce adverse actions on the cells (56).

Much more, there is evidence that TCS exposure decreases thyroid hormone concentrations in a dose-dependent manner because it chemically mimics the thyroid hormone. Thus, testicular alterations induced by TCS could be attributed to disruption of thyroid hormone homeostasis (57).

The ovaries of TCS-treated group revealed vacuolation of the granulosa cells around the Graafian follicles with absent ova and dilated antrum. The changes found in the ovaries were consistent with other study showing the same changes in ovaries (58).

TCS reduces thyroid hormones causing hyperprolactinemia, leading to deficits in reproductive endocrine and function. The numbers of antral follicles and corpora lutea were affected by the TCS exposure, where there was a significant reduction in the number of antral follicles and corpora lutea. Thus, it is conceivable that TCS-induced hyperprolactinemia may impair the follicle development and ovulation (59).

On the contrary, no marked changes or histology alterations occurred following chronic exposure in another study. However, there is an evidence that

exposure to estrogen-like compounds disrupt estrous cyclicity in the rat by impairing hypothalamic–pituitary–gonadal (HPG) axis regulation (60).

Other Potential mechanisms include signaling pathways that involve nuclear receptors, regulators of drug metabolizing, cell cycle, apoptosis, and steroidogenesis (61).

In Conclusion:

- 1- Triclosan caused low levels of sex hormones: testosterone, estrogen, FSH and LH.
- 2- It affected sperms count and motility.
- 3- It also caused histological and ultrastructures changes in testes and ovaries

Recommendations:

We urge the government to:

- A- Reconsider a regulatory tool that would prohibit and ban the use of triclosan in consumer products.
- B- Add triclosan to the List of Toxic Substances in the Egyptian Environmental Protection Act.
- C- Require informed substitution (including the option of omitting antimicrobial additives) by applying alternative assessments, to avoid regrettable substitutions to triclosan, to address potential contributions to antimicrobial resistance, and to ensure safe substitutes for triclosan.

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