THE PROTECTIVE ROLE OF "*CLEOME DROSERIFOLIA*" EXTRACT AGAINST TESTICULAR TOXICITY INDUCED BY LAMBDA-CYHALOTHRIN IN RATS

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ABSTRACT

Lambda-cyhalothrin (LCT) is pyrethroid insecticide that is used worldwide for pest control in agriculture and household use. *Cleome droserifolia* (CDE) extract is a potential antioxidant protecting cells from oxidative stress. The present study investigates the protective effect of CDE on LCT-induced changes in male rats. Forty adult male albino rats were divided into four groups: group I: served as control; group II: received LCT (6.23 mg/kg b.w.,i.p.) only; group III: received CDE (100 mg/kg b.w.,p.o.) only; group IV: received both CDE and LCT. LCT-intoxication elicited significant declines in serum testosterone, total protein and albumin, high density lipoprotein (HDL), whereas, serum

follicle stimulating hormone (FSH) and luteinizing hormone (LH), total cholesterol, triglycerides (TG), low density lipoprotein (LDL) and very low density lipoprotein

(VLDL) levels were significantly increased. Furthermore, LCT brought about significant disturbance in cytokines; tumor necrosis factor (TNF-α), interleukin-10(IL-10) andinterleukin-12(IL-12) levels. Testicular glutathione (GSH) content and superoxide dismutase (SOD) activity reduced significantly, while, testicular malondialdehyde (MDA) level was significantly elevated after LCT administration as compared to the control group. Histological and ultrastructural changes were also in accord with the above aberrations. However, treatment with CDE significantly attenuated LCT-induced changes in clinical chemistry parameters as well as it protected testicular tissues and cell structures. In conclusion, CDE has a potential protective role in ameliorating testicular toxicity induced by LCT in albino rats.

KeyWords: Lambda-cyhalothrin, *Cleome droserifolia*, Testis, Rat

INTRODUCTION

Lambda-cyhalothrin (LCT), a new generation type II synthetic pyrethroid insecticide, has extensive uses as an agro-pesticide (Fetoui et al., 2009). It is commonly used in Egypt and valued for its broad-spectrum control on a wide range of pests in a variety of applications (Abdel Aziz and Abdel Rahem, 2010). LCT has been found to accumulate in biological membranes leading to oxidative damage, it was reported that LCT caused oxidative stress by altering antioxidant systems and increasing lipid per-oxidation in mammals (Fetoui et al., 2008, 2009; Madkour, 2012). The production of reactive oxygen species (ROS) is a normal physiological event in various organs including testis

controlling sperm capacitation, acrosome reaction and sperm-oocyte fusion. However, over-production of ROS can be harmful to sperm and subsequently to male fertility (Akiyama, 1999).Although the organism has several biological defense mechanisms against intracellular oxidative stress including enzymatic and non-enzymatic antioxidant defense system, and can act to overcome the oxidative stress (Halliwell, 2006),a positive correlation has been established between dietary supplementation with certain vegetables and plant products and the reduction of toxic effects of various toxicants and environmental contaminants (Nandi et al., 1997).

Cleome droserifolia (CDE), family Capparaceae, commonly grow in different areas of North Sinai, Egypt.*Cleome* species are generally used in folk medicine as stomachics, rubefacients and in the therapy of scabies, rheumatic fever and inflammation (EL-Shenaway et al., 2006). The dried herb of CDE known as Samwah, Afein, Reeh-El-Bard, is used by herbalists in Egypt as a hypoglycemic agent, and its decoction is widely used in Sinai by Bedouins for the treatment of diabetes mellitus (El-Askary, 2005). Extract of leaves and stems of CDE is rich in bioactive compounds as flavonoids, glycosides, alkaloids, tannins and steroids (Nagy and Mohamed, 2014). Flavonoids from CDE were identified as quercetin, kaempferol, isorhamnetin, rutin and luteolin-7-O-glucoside (Abdel Motaal et al., 2011;Aparadh et al., 2012). Evidence suggests that certain phytochemicals found in citrus sources, such as flavonoids and limonoids, play an essential role in treating or retarding a wide spectrum of diseases and reported to possess anti-oxidative, anti-atherosclerotic, anti-inflammatory, antitumor, anti-thrombogenic, antiosteoporotic and antiviral properties (Nijveldt et al., 2001). Antioxidants protect deoxyribonucleic acid (DNA) and other important molecules from oxidation and damage,

can improve sperm quality and consequently increase fertility rate in men (Yang et al., 2006).

The objective of the current study was to analyze the testicular toxicity caused by Lambda-cyhalothrin (LCT) in male rats and to evaluate the possible protective effect of *Cleome droserifolia* extract (CDE), as an antioxidant, in alleviating the detrimental effect of LCT on male fertility.

MATERIAL AND METHODS

Animals

Forty adult male Wistar albino rats (weighing 120±10 g/animal, 7-8 weeks age) were obtained from the Animal House of Research Center, Faculty of Kasr Al-Ainy Medicine, Cairo University. The animals were housed under standard conditions of temperature (23±2°C), relative humidity (55±10%), and 12h light/12h dark cycle and were given food and water *ad libitum*. All ethical considerations for the studies on animals were considered carefully and the experimental protocol was approved by the Ethic Committee of Research on Laboratory Animals of Cairo University.

Lambda-cyhalothrin

Lambda-cyhalothrin (LCT) with the empirical formula ($C_{23}H_{19}ClF_3NO_3$) was used. It was obtained from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). LCT was dissolved in olive oil and administrated intraperitoneally (i.p.) at a dose of 1/10 LD_{50} (6.23 mg/kg/b.w.) (Fetoui et al., 2015).

Natural antioxidant *(Cleome droserifolia)*

The raw material was collected from Arish, North Sinai, Egypt, and authenticated to CDE by Agricultural Researches Center, Giza, Egypt. The CDE herb was spread over the bench and left for drying in the shade, then reduced to a powder. A decoction of the plant material was prepared by boiling 200 g of the dry plant material in 3 liters of tap water for 2 minutes and then filtered after 10 minutes. To minimize the volume of the decoction, it was concentrated in a rotary vacuum evaporator at a temperature below 40°C. The dried extracted material was stored at -20°C in clean vials until used (El-Khawaga et al., 2010). For oral administration (p.o.), the dried extract was dissolved in distilled water daily and administered by an intragastric tube at a dose of 100 mg/kg/b.w. (El-Naggar et al., 2005).

Experimental design

Rats were divided into four groups (each 10 animals) as follows: Group I: control, which received vehicles of LCT (olive oil) and CDE (distilled water) parallel to the treated groups, throughout the course of the study of 8 weeks; Group II: LCT group, which was given LCT (6.23 mg/kg b.w.,i.p.) only three times a week for two weeks; Group III: CDE group, in which the animals received CDE (100 mg/kg b.w.,p.o.) only in distilled water daily for eight weeks; Group IV: animals were given CDE (100 mg/kg b.w., p.o.) daily for eight weeks. On the 6th week just after CDE treatment they received LCT (6.23 mg/kg b.w., i.p.) three times a week for two weeks. At the end of the experimental period, blood samples were collected from the medial retro-orbital venous plexuses under ether anesthesia. Blood was centrifuged at 3000 rpm for 15 min and serum was collected for different clinical analysis. All animals were sacrificed and the testes were immediately

excised, washed in ice-cold saline, one of testis was separated and used for microscopical examination. Serum and the other testis samples were stored at -20°C until analysis.

Clinical pathology

Hormone measurements

Serum testosterone was measured using solid phase radioimmunoassay (RIA) kits. This assay based on testosterone-specific antibody immobilized to the wall of a poly-propylene tube according to Jaffe and Behrman (1974). Follicle stimulating hormone (FSH) and luteinizing hormone (LH) were estimated depending on the assays depicted by Santner et al. (1981) and Nankinand Troen (1971), respectively.

Determination of serum protein profile

Total protein and albumin levels in serum were determined colorimetrically according to Henry et al. (1974) and Doumas et al. (1971), respectively.

Determination of serum lipid profile

Serum cholesterol and triglycerides (TG) levels were measured enzymatically by the methods of Seidel et al. (1983) and Fossati and Prencipe (1982), respectively, and high-density lipoprotein (HDL) level by using the method of Stein (1986). The levels of low-density lipoprotein (LDL)and very low-density lipoprotein (VLDL) were estimated according to the equation of Assmann et al. (1984).

Determination of cytokine levels

Serum tumor necrosis factor (TNF-α), interleukin-10 (IL-10) and interleukin-12 (IL-12) were determined according to the method of Aggarwal and Natarajan (1996); Okura et al. (1998) and Trinchieri (1998), respectively.

Determination of reduced glutathione (GSH) and enzymatic antioxidant (SOD)

Testicular glutathione (GSH) content was assayed spectrophotometrically by the method of Tietze (1969). The activity of superoxide dismutase (SOD) was determined by assessing the inhibition of pyrogallol auto-oxidation (Nishikimi et al., 1972).

Determination of oxidative stress markers (MDA)

Testicular malondialdehyde (MDA) was measured spectrophotometrically after the reaction with thiobarbituric acid (TBA) by using the method of Botsoglou et al. (1994).

Histopathological and ultrastructural examinations

For light microscopic examination, the testicular specimens were fixed in 10% neutral buffered formalin. After a proper fixation for 48h, the tissue was cut into thinner pieces. The samples were dehydrated, cleared, embedded in paraffin, sectioned on a rotary microtome at 4-5 µm thickness, and stained using hematoxylin and eosin (H&E) method described by Drury and Wallington (1980). Stained sections were viewed under a light microscope.

For transmission electron microscopic study, small pieces (about $1mm^3$) from the testis were rapidly cut and immediately fixed in 5% glutaraldehyde buffered at pH 7.2 with 0.1M sodium cacodylate for 4 hours at room temperature. Specimens were then post fixed in 2% osmium tetroxide for 1h at 4°C, washed three times for15 min each time with 0.1M sodium cacodylate buffer and dehydrated in ascending ethyl alcohol series. Dehydrated specimens were infiltrated at room temperature in a mixture of propylene oxide/ epoxy resin and then embedded in fresh resin and polymerized at 60°C for 48 hours. Semi-thin sections (1µm) were cut by an ultramicrotome (Leica Ultracut UCT; Germany) using a glass knife, stained with 1% toluidine blue for selection of adequate

fields under a light microscope. Ultrathin sections (60-80 nm thickness) of the selected areas were cut using a diamond knife, transferred to copper grids and followed by double-staining with uranyl acetate and lead citrate. The grids were examined and photographed using a JEOL transmission electron microscope (JEM-1010, Japan) operated at 60-70 kV accelerating voltage, Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University.

Statistical analysis

All data were analyzed using the SPSS for windows software, version 17.0 statistical program. Analysis of variance (ANOVA) which is an indication of the dispersion or difference between more than two means to the calculated standard error of this difference was assessed (Tello and Crewson, 2003).

RESULTS

In rats treated with CDE only (group III) for eight weeks, no differences were recorded between this group and the control one.

Clinical pathology

Effect of CDE on LCT-induced alteration in serum sexual hormones

Testosterone, FSH and LH hormones levels from rats exposed to LCT and/or CDE are presented in Table 1. There were differences in the hormones among the different treated-groups. LCT significantly ($p<0.001$) decreased serum level of testosterone (-78.96%) while, elevated the level of FSH and LH hormones (42.64% and 52.60%, respectively) in group II compared to control rats (group I). Whereas, group IV significantly preserve these aforementioned changes to near normal levels as compared with that of LCT-

treated rats (group II). The percentages of change in group IV were -7.29%, 9.43% and 1.56% for testosterone, FSH and LH, respectively as compared to the control group.

Effect of CDE on LCT-induced alteration in protein profile

Serum total protein and albumin level are given in Table 1. In relation to the control rats (group I), a very highly significant decrease in total protein and albumin level (-43.25% and -62.18%, respectively) were reported in rats treated with LCT (group II). Furthermore, administration of CDE before administration of LCT (group IV) recovered the levels of serum protein and albumin close to normal. This change reached -11.56% in total protein and -21.05% in albumin as compared to control group (group I).

Effect of CDE on LCT-induced alteration in lipid profile

Table 2 illustrated that the serum cholesterol, TG, LDL and VLDL were significantly ($p<0.001$) elevated in LCT rats(group II) reached to 69.40%, 63.80%, 53.19% and 63.85%, respectively, while HDL recorded -46.91% as compared with control rats(group I). A very highly significant improvement was observed in serum total cholesterol, TG, HDL, LDL and VLDL concentrations in group IV as compared to the control group (group I). The percentages of change in those parameters were -1.83%, -0.48%, -3.31%, -2.87% and -0.46%, respectively.

Effect of CDE on LCT-induced alteration in cytokines

Data recorded for the levels of serum TNF-α, IL-10 and IL-12 were presented in Table 3. LCT group (group II) exhibited a very highly significant elevation of TNF-α and IL-12 levels (227.03% and 119.62%, respectively), but a very highly significant decrease of IL-10 level (-81.26%) in group II as compared to control group (group I). The

administration of CDE before treatment with LCT in group IV significantly ($p<0.001$) preserved the levels of TNF-α, IL-10 and IL-12 to near normal level when compared with LCT group (group II). The percentages of change were 40.95%,-10.21% and 20.17%, respectively as compared to control group (group I).

Effect of CDE on LCT-induced alteration in antioxidants and oxidative stress

Table 3. presents the testicular GSH content, SOD activity and MDA level in both normal and treated groups. In LCT rats (group II), a very highly significant depletion in the content of tissue GSH and activity of SOD were recorded (-46.80% and -53.04%, respectively) and a very highly significant elevation was realized in tissue MDA level (95.31%) in group II as compared with the control rats (group I). However, these parameters in group IV were nearly similar to that in group I and recorded -15.27%, -12.86% and 26.56% for testicular GSH, SOD and MDA, respectively as compared to normal rats (group I).

Microscopic studies

Light microscopic study

Evaluation of hematoxylin and eosin-stained testicular sections of control rats (group I) and CDE-treated rats (group III) revealed alike normal histological structure of the testis. The parenchyma is formed of densely packed seminiferous tubules, with little interstitium. The seminiferous tubules are lined by several layers of germinal epithelium formed of spermatogonia, primary spermatocytes, secondary spermatocyte, early round and late elongated spermatids. Sertoli cells appear as elongated cells with a pale cytoplasm and oval intended nuclei in between the spermatogenic cells (Figure 1). The tubules are surrounded by a layer of flat peritubular cells, and the lumina of the tubules

contain mature spermatozoa (Figure 1). The interstitial tissue contains interstitial cells of Leydig and small capillaries, in addition to fibroblasts and macrophages (Figure 1).

Inspection of testis sections of rats of LCT group (group II) revealed severe damage of the seminiferous tubules with separating of cells from underlying layer of the tubules, exfoliation and depletion of germ cell leaving empty spaces (vacuoles) and hyalinization of the luminal contents (Fig. 2a & b). Edema and widening of the intertubular spaces together with a diminution of the stromal interstitial tissue were also illustrated (Figure 2a & b).

In rats treated with CDE (group IV) as a protective agent then followed by the administration of LCT, the histopathological changes were greatly reduced. This group showed recovery and preservation of seminiferous epithelium except separating of few cells from underlying layer and appearance of intraepithelial vacuoles and few degenerated spermatogenic cells (Figure 3a & b).

Transmission electron microscopic study

Electron microscopic examination of the ultrathin sections of the testis of control rats (group I) and CDE-treated rats (group III) revealed that each seminiferous tubule is coated with boundary tissue which consists of the thin basal lamina, the inner non-cellular layer, the inner cellular layer, the outer non-cellular layer and the outer cellular layer. Spindle shaped smooth muscle cells (myoid cells) is the composition of the inner cellular layer (Figure 4a). Sertoli cells have large pale nuclei and prominent nucleoli. Spermatogonia appear with rounded nuclei resting on the basal lamina. Spermatocytes have large euchromatic nuclei with a thin rim of cytoplasm containing mitochondria (Figure 4a). Early differentiating spermatid contained numerous peripherally arranged

mitochondria and developing acrosomes that appeared as acrosomal granules and acrosomal vesicles spreading over a rounded nucleus (Figure 4b). Near the lumen, elongated late spermatids with strongly elongated condensed nuclei covered anteriorly by the acrosome cap are detected (Figure 4c).

Electron microscopic examination of ultrathin sections of the rat testis of LCT group (group II) revealed that Sertoli cell does not rest on the basement membrane but is separated from it by a part of cytoplasm (Figure 5a). The cytoplasm of Sertoli cell, spermatogonia, and primary spermatocytes is highly degenerated and contained smooth endoplasmic reticulum-derived vacuoles along with disintegration or overall decreases of cytoplasmic organelles (Figure 5a & 5b). Irregular, infolded boundary tissue of seminiferous tubules and numerous variable lipid globules were also observed in Sertoli cells (Figure 5b). The nuclear chromatin of some spermatogonia and spermatocyte is clumped and more electron dense with a reduction in the size of the cells (Figure 5b). Deformed spermatids with irregular outlines, disorganized nuclei with darkly stained heterochromatin and distorted acrosomes, in addition to degenerated mitochondria were also evident (Figure 5c). Cellular debris could be seen in the tubular lumina (Figure 5b).

The ultrastructure of the seminiferous tubules of the rat testis in the group treated with CDE as a protective agent then followed by the administration of LCT (group IV) showing a nearly normal structure of boundary tissue and relatively unaffected spermatogonia and spermatocytes. Almost all the mitochondria appear normal, except fusion of few ones (Fig. 6a). Also, most of the spermatids appeared normal with its characteristic acrosomal vesicles (Figure 6b). However, increased lipid globules were still observed in Sertoli cells (Figure 6a).

DISCUSSION

Lambda-cyhalothrin (LCT), a type II pyrethroid, is widely used for numerous applications, varying from plant protection to general pest control. Improper use of this agent can potentially lead to adverse effects in multiple organs. This study was conducted to evaluate the role of *Cleome droserifolia* extract (CDE) in attenuating testicular damage induced by Lambda-cyhalothrin (LCT).

The present study showed a marked decrease in the levels of serum testosterone and a significant increase in the levels of serum FSH and LH in rats exposed to LCT (group II). This may be due to disruption of the feedback mechanisms existing between hypothalamic-pituitary-gonadal axes, a decline in the number of viable steroidogenic cells in the testis and the effect of toxicant in the testicular cells. These results are in agreement with Oda and El-Maddawy (2011) who observed a significant reduction of serum testosterone level in deltamethrin (a type II synthetic pyrethroid insecticide)-treated rats.

Moreover, decreased testosterone synthesis might be associated with down-regulation of steroidogenic acute regulatory protein (StAR) in testis. StAR is essential in testosterone synthesis in Leydig cells. StAR is responsible for the transport of cholesterol in the inner mitochondrial membrane of Leydig cells (Miller, 2007),where testosterone synthetic enzymes, primarily P450scc, $P450_{17\alpha}$ and 17β-HSD, play a critical role in testosterone synthesis in Leydig cells. P450scc initiates the first enzymatic step in testosterone biosynthesis as cholesterol is converted to pregnenolone. Pregnenolone is catalyzed by $P450_{17\alpha}$to produce 17-hydroxyprogesterone and androstenedione, with the latter then

being converted to testosterone by 17β-HSD (Payne and Youngblood 1995). Also, these results are in agreement with an earlier study, in which plasma testosterone was markedly decreased in rabbits chronically exposed to cypermethrin, a widely used synthetic pyrethroid insecticide, for 12 weeks (Yousef et al. 2003). Additionally, Zhang et al. (2007) showed that the exposure of adult mice to permethrin, another pyrethroid insecticide, disrupted testicular testosterone biosynthesis *via* inhibiting the mRNA and protein expression of StAR in testes. More recently, Wang et al.(2010) investigated the effects of cypermethrin exposure during puberty on the expression of StAR in testes of adult mice. They showed that mRNA level of testicular StAR was significantly decreased in cypermethrin-treated mice. Testosterone, FSH and LH hormones were improved significantly in the protective group treated with CDE (group IV). The increase in testosterone level in the protective was supported by Helal et al. (2002) who showed that the observed high testosterone level in CDE-treated diabetic rat is expected as a result of an increase in leptin which potentially plays an important role in the control of the reproductive function (Cervero et al., 2006). Moreover, quercetin the major active antioxidant flavonoids in CDE, stimulated steroid hormone synthesis in MA-10 mouse tumor Leydig cells through up-regulation of StAR promoter activity and mRNA expression (Chen et al., 2007). Also, quercetin attached to the iron ion Fe^{2+}, preventing it from binding to hydrogen peroxide, which create a highly-reactive free radical that may damage cells (Chow et al., 2005)

LCT induced depletion in total protein and albumin levels in the serum compared to control (group I). This may be attributed to the destruction/necrosis of cells and a reduction of the protein synthesis in the liver (Parthasarathy and Joseph, 2011). These

results are in agreement with Morgan and Osman (2007) who reported that LCT induces hepatotoxic effects in rabbits with a consequent suppressive effect on plasma protein production and/or albumin. Treatment with CDE in the protective group (group IV) showed very highly significant improvement in the levels of both albumin and total protein compared with LCT group (group II). This may be ascribed to the antioxidant capability of the extract. These observations are in accordance with El-Shenawyand Abdel-Nabi (2006) who attributed the significant increase in serum total protein and albumin in CDE-treated diabetic mice to an elevation in serum insulin level, consequently, accelerates amino acid transport through cells and stimulates the protein manufacturing machinery of the cell.

The present results elucidated high significant increase in serum cholesterol, TG, LDL, VLDL levels in LCT-treated rats (group II) and highly significant decreased HDL level as compared to control group (group I). These results in LCT intoxicated rats clearly reflect the abnormalities in lipoprotein metabolism (Abu-Aita and Yassa, 2008). Treatment of LCT intoxicated rat with CDE (group IV) induced marked significant decrease of serum cholesterol, TG, LDL, VLDL levels and showed high significant level of HDL as compared to LCT group (group II), which may be due to modulator influence on lipogenic enzymes or by inhibition of cholesterol absorption (Jain and Agarwal, 2006; El-Khawaga et al., 2010).

Administration of LCT (group II) resulted in disruption of some immune biomarkers as reflected by the significant increase in serum TNF-α and IL-12 and a significant decrease in IL-10 levels. These results are in harmony with Neta et al. (2011) who showed negative association between levels of permethrin insecticide and levels of anti-

inflammatory cytokine component. A significant improvement was observed in group IV in the same parameters (TNF-α, IL-10 and IL-12).This could be due to the presence of flavonoid, alkaloids and tannins in extract. Flavonoids have an anti-inflammatory, anti-mutagenic and anti-malignant effect (Fotsis et al., 1997; Bala et al., 2010).

In the present study, significant depletion in the tissue GSH content and SOD activity was designated, while a significant elevation was realized in tissue MDA level in LCT rats (group II) as compared with the control group (group I). LCT toxicity might be due to the release of cyanohydrins, which are unstable under physiological conditions and further decompose to cyanides and aldehydes which in turn could act as a source of free radicals. These results are consistent with the findings of other investigators (Madkour, 2012). Moreover, pesticides induce oxidative stress that leads to the generation of free radicals, changes in antioxidants levels and lipid per-oxidation. They generate various radicals such as superoxide radical (O^{2-}), and hydroxyl radical ($OH^{\cdot}$) thus causing failure of an antioxidant defense system to overcome the influx of ROS induced by LCT exposure (Fetoui et al., 2010). The improvement of GSH content and SOD activity and decreased lipid per-oxidation recorded after CDE treatment (group IV) might be mediated through neutralization of oxygen free radicals produced by LCT (El-Khawaga et al., 2010). Antioxidative properties of CDE can be explained by the presence of the glycosidic flavonoids as quercetin, rutin, kaempferol, luteolin, isorhamnetin and other phenolic acids (Aparadh et al., 2012).

Regarding the light microscopic examination, LCT administration (group II) provoked some histopathological changes in the testis such as degeneration, desquamation of necrotic cells and depletion of the germinal epithelium followed by hyalinization of the

luminal content. Also, diminution of the stromal interstitial tissue together with a widening of the intertubular spaces was observed. Similar results have been observed in rats (Mate et al., 2010; Oda and Maddawy, 2011), mice (Abdel Aziz and Abdel Rahem, 2010; Hussein et al., 2012; Al-Shaikh, 2013) and fish (Parthasarathy and Joseph,2011) after pyrethroid insecticides administration. In view of the fact that Sertoli cells are the supportive cells within the seminiferous tubule and provide multitude factors required for spermatogenesis (Russell, 1993). Consequently, sloughing of germ cells pointed out the Sertoli cell damage due to microtubule impairment (Kumar et al., 2006).Also, abnormal exfoliation of the degenerated germ cells could be due to a primary effect on the cell-to-cell junctions between Sertoli and germ cells. Moreover, the spaces and vacuoles detected in between the spermatogenic epithelium might be because of the exfoliation and the sloughing of germ cells (Creasy, 2001). Additionally, diminution of Leydig cells observed in the present study may be due to vascular congestion and edema in its surrounding environment. These changes could be the cause of testosterone shortage (Al-Shaikh, 2013). Wherein, testosterone is required for the attachment of different generations of germ cells in seminiferous tubules. Therefore, low level of intra-testicular testosterone may lead to detachment of germ cells from seminiferous epithelium and may initiate germ cell apoptosis and subsequent male infertility (Blanco-Rodríguez and Martínez-García, 1998).

In this regard, Abdel Aziz and Abdel Rahem (2010) observed chromosomal abnormalities and a reduction of the meiotic division after LCT treatment. This could be explained by two reasons: the first is the ability of the insecticide to reduce DNA synthesis, which subsequently affects cell division (Sotomayor et al., 2003). The second

reason is the toxic effect of the substance under test which affects the rate of cell division (Verma and Nair, 2001).

By electron microscopy, as a consequence of LCT administration (group II) the seminiferous tubules showed deformed and irregular basal laminae, abnormal spermatogenic cells, deformed spermatids, in addition to highly degenerated and vacuolated Sertoli cell with an overall decrease in cytoplasmic organelles. Similar findings were also recorded in testis of mice after administration of cypermethrin pyrethroid insecticide (Al-Shaikh, 2013).Also, Wang et al., (2009) showed that thickening of basement membrane due to beta-cypermethrin exposure could play a role in germ cell atrophy and death related to interference with germ cell nutrition. Moreover, vacuolization of the germinal cells and Sertoli cells were previously hypothesized to be derived from dilatation and vesiculation of the smooth endoplasmic reticulum (Murthy et al., 1991). This dilatation might be due to the ingress of water into the cell as a part of hydropic degeneration. In addition, Creasy (2001) revealed that vacuolations of Sertoli cells were an early and common morphological response to injury. Sertoli cells play a very important role in spermatogenesis by regulating the immediate environment of the developing germ cells, by providing physical support, junctional complexes or barriers, and/or biochemical stimulation in the form of growth factors or nutrients. Thus, Sertoli cell damage observed in the present study may lead to an insufficient supply of nutrients to spermatogenic cells, which may lead to structural alterations observed in spermatogenic cells.

In the present investigation, the structural alteration may be attributed to LCT-induced lipid peroxidation (LPO). In this respect, Piner andÜner (2012) reported that LTC

metabolism generates reactive oxygen species (ROS), these radicals can destroy proteins; lipids and DNA by oxidation and attack the cell membrane leading to destabilization and disintegration of cell membrane and decrease its fluidity as a result of LPO and protein oxidation which in turn leads to enhanced LPO and oxidative stress. Sperm is highly susceptible to LPO as a result of the abundance of unsaturated fatty acids in the sperm plasma membrane and a very low concentration of cytoplasmic antioxidants (Aitken et al., 1993). The increased LPO can lead to oxidative damage to sperms DNA, alter membrane functions, impair motility and possibly have a significant effect on the development of spermatozoa (Aitken et al., 1989).

The concurrent histological and ultrastructural findings show significant protective effects of CDE in LCT-induced testis damage in rats (group IV). This might be due to accelerated regeneration of parenchymal cells under the influence of various bioactive compounds like flavonoids present in CDE, mainly quercetin (Abdel Motaal et al., 2011; Ezzat and Abdel Motaal, 2012). Quercetin, a natural antioxidant, exists as the active substance in CDE (Aboushoer et al.,2010; Ezzat and Abdel-Motaal, 2012).Being a strong antioxidant, quercetin shows attenuating effects contrary to reproductive toxicity *via* decreasing lipid peroxidation, restoring the antioxidant status of cells and allaying apoptosis by regulating the expression of Bax and (B-cell lymphoma-extra large) Bcl-xl, caspase-3 activity (Mi et al., 2013). Also, vitamin C is considered an antioxidant in the *Cleome* species (Jain and Agarwal, 2006; Bala et al., 2010). It neutralizes ROS and prevents sperm agglutination (Dawson et al., 1992). It is a donor of electrons for redox systems, prevents lipid peroxidation, recycles vitamin E and protects against DNA damage induced by hydrogen peroxide radical (Jedlinska-Krakowska et al., 2006).

Tremellen (2008) observed a significant correlation between vitamin C intake and sperm concentration. In addition, the non-enzymatic antioxidants present within semen include ascorbic acid. Also, Narayana et al. (2009) declared that vitamin C significantly, but not completely, protects against cisplatin-induced testicular damage. Moreover, Fernandes et al. (2011) showed that vitamin C supplementation minimized some alterations in the male reproductive system caused by hyperglycemia such as reduction of testosterone and LH levels and impairment in sperm morphology. B-cell lymphoma-extra large

In conclusion, the results of the present investigation (clinical pathology, histopathology, and ultrastructure) demonstrate that the water extract of CDE at the dose of 100 mg/kg displayed a protective effect against LCT-induced reproductive toxicity in male albino rats *via* reducing oxidative stress. Such effects can be explained, at least in part, by the presence of the previously mentioned flavonoids. However, further clinical studies are necessary to assess the benefits and safety of CDE before using for human beings.

CONFLICT OF INTEREST

The authors declared no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

Abdel Aziz KB, Abdel Rahem HM. (2010). Lambda, the pyrethroid insecticide as a mutagenic agent in both somatic and germ cells. J Am Sci, 6, 317-326.

Abdel Motaal A, Ezzat SM, Haddad PS. (2011). Determination of bioactive markers in *Cleome droserifolia* using cell-based bioassays for antidiabetic activity and isolation of two novel active compounds. Phytomedicine, 19, 38-41.

Aboushoer MI, Fathy HM, Abdel-Kader MS, Goetz G,Omar AA.(2010). Terpenes and flavonoids from an Egyptian collection of *Cleome droserifolia*.Nat Prod Res, 24, 687-696.

Abu-Aita NA, Yassa VF. (2008). Impact of vitamin C on genotoxicity, sperm abnormalities and serum biochemical alterations in deltamethrin exposed rats. Egypt J Comp Path Clinic Path, 1, 168-188.

Aggarwal BB, Natarajan K. (1996). Tumor necrosis factor: developments during last decade. Eur Cytokine Netw, 7, 93-124.

Aitken RJ, Clarkson JS,Fishel S. (1989). Generation of reactive oxygen species, lipid peroxidation, and human sperm function.Biol Reprod, 41, 183-197.

Aitken RJ, Harkiss DU, Buckingham DW. (1993). Analysis of lipid peroxidation mechanisms in human spermatozoa.MolReprod Dev, 35, 302-315.

Akiyama M. (1999). *Invivo* scavenging effect of ethylcysteine on reactive oxygen species in human semen. Nihon Hinyokika Gakkai Zasshi, 90, 421-428.

Al-Shaikh TM. (2013). The study of the protective effect of vitamin E against cypermethrin toxicity on testicular histology in mice. J Am Sci, 9, 615-621.

Aparadh VT, Mahamuni RJ, Karadge BA. (2012). Taxonomy and physiological studies in spider flower (*Cleome* species): A Critical Review. Plant Sciences Feed, 2,25-46.

Assmann G, Jabs HU, Kohnert U, Nolte W, Schriewer H. (1984). LDL-cholesterol determination in blood serum following precipitation of LDL with polyvinyl sulfate.Clin Chem Acta, 140, 77-83.

Bala A, Kar B, Haldar PK, Mazumder UK, Bera S. (2010). Evaluation of anticancer activity of *Cleome gynandra* on Ehrlich's Ascites Carcinoma treated mice. J Ethnopharmacol, 129, 131-134.

Blanco-Rodríguez J,Martínez-García C. (1998).Apoptosis precedes detachment of germ cells from the seminiferous epithelium after hormone suppression by short-term oestradiol treatment of rats.Int J Androl, 21, 109-115.

Botsoglou NA, Fletouris DJ, Papageorgiou GE, Vassilopoulos VN, Mantis AJ,Trakatellis AG. (1994). Rapid sensitive and specific thiobarbituric acid method for measuring lipid peroxidation in animal tissue, food and feed stuff samples.J Agric Food Chem, 42, 1931-1937.

Cervero A, Domínguez F, Horcajadas JA, Quiñonero A, Pellicer A, Simón C. (2006).The role of the leptin in reproduction.Curr Opin Obstet Gynecol,18, 297-303.

Chen YC,Nagpal ML, Stocco DM, Lin T. (2007). Effects of genistein, resveratrol, and quercetin on steroidogenesis and proliferation of MA-10 mouse Leydig tumor cells. J Endocrinol, 192, 527-537.

Chow JM, Shen SC, Huan SK, Lin HY, Chen YC. (2005): Quercetin, but not rutin and quercitrin, prevention of H2O2-induced apoptosis via anti-oxidant activity and heme oxygenase 1 gene expression in macrophages. Biochem.Pharmacol, 69, 1839-1851.

Creasy DM. (2001).Pathogenesis of male reproductive toxicity.ToxicolPathol, 29,64-76.

Dawson EB, Harris WA, Teter MC, Powell LC. (1992). Effect of ascorbic acid supplementation on the sperm quality of smokers.Fertil Steril, 58, 1034 -1039.

Doumas BT, Watson WA, Biggs HG. (1971). Albumin standard and the measurement of serum albumin with bromocresol green. Clin Chem Acta, 31, 87-96.

Drury RA, Wallington EA. (1980). Carleton's Histological Techniques.5th Edition. New York: Oxford University Press, 126-133.

El-Askary HI. (2005). Terpenoids from *Cleome droserifolia* (Forssk.) Del. Molecules, 10, 971-977.

El-Khawaga OAY, Abou-Seif MA, El-Waseef A, Negm AA. (2010). Hypoglycemic, hypolipidemic and antioxidant activities of *Cleome droserifolia* in streptozotocin-diabetic rats.Journal of Stress Physiology & Biochemistry, 4, 28-41.

El-Naggar EMB, Bartosikova L, Zemlicka M, Svajdlenka E, Rabiskova M, Strnadova V, Necas J. (2005). Antidiabetic effect of *Cleome droserifolia* aerial parts: lipid peroxidation-induced oxidative stress in diabetic rats. Acta Vet Brno, 74, 347-352.

El-Shenawy NS, Abdel-Nabi IM. (2006). Hypoglycemic effect of *Cleome droserifolia*ethanolic leaf extract in experimental diabetes, and on non-enzymatic antioxidant, glycogen, thyroid hormone and insulin levels. Diabetologia Croatica, 35,15-22.

Ezzat SM, Abdel Motaal A. (2012). Isolation of new cytotoxic metabolites from *Cleome droserifolia*growing in Egypt. Z Naturforsch C, 67, 266-274.

Fernandes GS, Fernandez CD, Campos KE, Damasceno DC, Anselmo-Franci JA, Kempinas WD. (2011). Vitamin C partially attenuates male reproductive deficits in hyperglycemic rats. Reprod Biol Endocrinol, 9, 100.

Fetoui H, Garoui el M, Makni-Ayadi F, Zeghal N. (2008). Oxidative stress induced by lambda-cyhalothrin (LTC) in rat erythrocytes and brain: attenuation by vitamin C. Environ Toxicol Pharmcol, 26, 225-231.

Fetoui H, Garouiel M, Zeghal E. (2009). Lambda-cyhalothrin-induced biochemical and histopathological changes in the liver of rats: ameliorative effect of ascorbic acid. Exp Toxicol Pathol, 61, 189-196.

Fetoui H, Makni M, Garoui M, Zeghal N. (2010). Toxic effects of lambda-cyhalothrin, a synthetic pyrethroid pesticide, on the rat kidney: Involvement of oxidative stress and protective role of ascorbic acid. Exp Toxicol Pathol, 62, 593-599.

Fetoui H, Feki A, Salah GB,Kamoun H, Fakhfakh F, Gdoura R. (2015). Exposure to lambda-cyhalothrin, a synthetic pyrethroid, increases reactive oxygen species production and induces genotoxicity in rat peripheral blood. Toxicol Ind Health, 31,433-441.

Fossati P, Prencipe L. (1982). Serum triglycerides determined calorimetrically with an enzyme that produces hydrogen peroxide. Clin Chem, 28, 2077-2080.

Fotsis T, Pepper MS, Aktas E, Breit S, Rasku S, Adlercreutz H, Wähälä K, Montesano R, Schweigerer L. (1997). Flavonoid, dietary-derived inhibitors of cell proliferation and *in vitro* angiogenesis. Cancer Res, 57, 2916-2921.

Halliwell B. (2006). Oxidative stress and neurodegeneration: where are we now? Journal of Neurochem, 97, 1634-1658.

Helal EGE, Sharaf HA, Mattar FE. (2002). Anti-diabetic and anti-obesity effects of plant extract from *Cleome droserifolia* (SAMWA). The Egyptian Journal of Hospital Medicine, 9, 85-101.

Henry RJ, Cannon DC, Winkelman JW. (1974). Quantitative colorimetric determination of total protein in serum. Clinic Chem Prin Tech, 2nd Edition, New York, Harper and Row, 411-421.

Hussein HK, El-Naggar MH, Al-Zahrani NK. (2012). Antioxidant role of folic acid against reproductive toxicity of cyhalothrin in male mice.Global Advanced Research Journal of Environmental Science and Toxicology,1, 066-071.

Jaffe BM, Behrman NR. (1974). Methods of hormone radioimmunoassay.Academic Press.

Jain GC, Agarwal S. (2006). Favourable effect of *Cleome viscosa L*. on serum and hepatic lipids in hyperlipidemic rats. Asian J Exp Sci, 2, 331-336.

Jedlinska-Krakowska M, Bomba G, Jakubowski K, Rotkiewicz T, Jana B, Penkowski A. (2006). Impact of oxidative stress and supplementation with vitamins E and C on testes morphology in rats. J Reprod Dev, 52, 203-209.

Kumar SG, Narayana K, Bairy KL, D'Souza UJ, Samuel VP, Gopalakrishna K. (2006). Dacarbazine induces genotoxic and cytotoxic germ cell damage with concomitant decrease in testosterone and increase in lactate dehydrogenase concentration in the testis. Mutat Res, 607, 240-252.

Madkour NK. (2012). Protective effect of curcumin on oxidative stress and DNA fragmentation against lambda cyhalothrin-induced liver damage in rats.Journal of Applied Pharmaceutical Science, 2, 76-81.

Mate MS, Ghosh RC, Mondal S, Karmakar DB. (2010). Effect of lambda cyhalothrin on rats: an acute toxicity study. Journal of the Indian Society of Toxicology (JIST),006, 25-28.

Mi Y, Tu L, Wang H, Zeng W, Zhang C. (2013). Supplementation with quercetinattenuates 4-nitrophenol-induced testicular toxicity in adult male mice. Anat Rec (Hoboken), 296, 1650-1657.

Miller WL. (2007). StAR Search-What we know about how the steroidogenic acute regulatory protein mediates mitochondrial cholesterol import. Mol Endocrinol, 21, 589-601.

Morgan AM, Osman AH. (2007). Immunotoxic effects of lambda-cyhalothrin in rabbits. J Egypt Soc Toxicol, 36, 23-33.

Murthy RC,Saxena DK, Gupta SK, Chandra SV. (1991). Lead induced ultrastructural changes in the testis of rats. ExpPathol, 42, 95-100.

Nagy MA, Mohamed SA. (2014). Antidiabetic effect of *Cleome droserifolia* (Cleomaceae). Am J Biochem, 4, 68-75.

Nandi P, Talukder G, Sharma A. (1997). Dietary factor in cancer chemoprevention. The Nucleus, 40, 128-144.

Nankin HR, Troen P.(1971). Repetitive luteinizing hormone elevation in serum of normal men. J Clin Endo Metab, 33, 558-560.

Narayana K, Verghese S, Jacob SS. (2009). Ascorbic acid partially protects two cycles of cisplatin chemotherapy-induced testis damage and oligo-astheno-teratospermia in a mouse model. Exp Toxicol Pathol, 61, 553-563.

Neta G, Goldman LR, Barr D, Apelberg BJ, Witter FR, Halden RU. (2011). Fetal exposure to chlordane and permethrin mixtures in relation to inflammatory cytokines and birth outcomes. Environ Sci Technol, 45, 1680-1687.

Nijveldt RJ, van Nood E, van Hoorn DE, Boelens PG, van Norren K, van Leeuwen PA. (2001). Flavonoids: a review of probable mechanisms of action and potential applications.Am JClinNutr,74, 418-425.

Nishikimi M, Appaji N, Yagi K. (1972). The occurrence of superoxide anion in the reaction of reduced phenazinemethosulfate and molecular oxygen. Biochem Biophys Res Commun, 46, 849-854.

Oda SS, El-Maddawy ZK. (2011). Protective effect of vitamin E and selenium combination on deltamethrin-induced reproductive toxicity in male rats.Exp Toxicol Pathol, 64, 813-819.

Okura Y, Takeda K, Honda S, Hanawa H, Watanabe H, Kodama M, Izumi T, Aizawa Y, Seki S, Abo T. (1998). Recombinant murine interleukin-12 facilitates induction of cardiac myosin-specific type 1 helper T cells in rats. Circ Res, 82, 1035-1042.

Parthasarathy R, Joseph J. (2011). Study on the changes in the levels of protein metabolism in λ cyhalothrin-induced hepatotoxicity in fresh water *Tilapia (OreochromisMossambicus)*. Advances in Applied Science Research, 2, 57-62.

Payne AH, Youngblood GL. (1995).Regulation of expression of steroidogenic enzymes in Leydig cells. BiolReprod, 52, 217–225.

Piner P, Uner N. (2012). Oxidative and apoptotic effects of lambda-cyhalothrin modulated by piperonylbutoxide in the liver of Oreochromisniloticus. Environ Toxicol Pharmacol, 33, 414-420.

Russell LD. (1993). Role in spermiation. In: Russell LD, Griswold MD ed. The Sertoli cell. Clearwater, FL: Cache River Press.

Santner SJ, Santen RJ, Kulin HE, Dermers LM. (1981). A model for validation of radioimmunoassay kit reagents: measurement of fallitropin and lutropin in blood and urine. Clin Chem, 27, 1892-1895.

Seidel J, Hagele EO, Zingenhorn J,Wahlfeld AW. (1983). Reagent for the enzymatic determination of serum total cholesterol with improved lipolytic efficiency. Clin Chem, 29, 1075-1080.

Sotomayor RE, Wahington M, Nguyen L, Myang'anyi R, Hinton DM, Chou M. (2003). Effects of intermittent exposure to Aflatoxin B1 on DNA and RNA adduct formation in rat liver: dose-response and termporal patterns. Toxicol Sci, 73, 329-338.

Stein EA. (1986). Lipids, lipoproteins and apolipoproteins. In: Tietz NW, ed. Textbook of Clinical Chemistry, W. B. Sounders Co Philadelphia, 448-81.

Tello R, Crewson PE. (2003). Hypothesis testing II: means. Radiology, 227, 1-4.

Tietze F. (1969). Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. Anal Biochem, 27, 502-522.

Tremellen K. (2008). Oxidative stress and male infertility-- a clinical perspective. Hum Reprod Update, 14, 243-258.

Trinchieri G. (1998). Interleukin-12: a cytokine at the interface of inflammation and immunity. Adv Immunol, 70, 83-243.

Verma RJ, Nair A. (2001). Vitamin E ameliorates aflatoxin-induced biochemical changes in the testis of mice. Asian J Androl, 3, 305-309.

Wang H, Wang Q, Zhao X F, Liu P, Meng X H, Yu T, Ji YL, Zhang H, Zhang C, Zhang Y, Xu DX. (2010). Cypermethrin exposure during puberty disrupts testosterone synthesis *via* downregulating StARin mouse testes. Arch Toxicol, 84, 53-61.

Wang XZ, Liu SS, Sun Y, Wu JY, Zhou YL, Zhang JH. (2009). Beta-cypermethrin impairs reproductive function in male mice by inducing oxidative stress. Theriogenology, 72, 599-611.

Yang HS, Han DK, Kim JR, Sim JC. (2006). Effects of alpha-tocopherol on cadmium-induced toxicity in rat testis and spermatogenesis. J Korean Med Sci, 21, 445-451.

Yousef MI, El-Demerdash FM, Al-Salhen KS. (2003). Protective role of isoflavones against the toxic effect of cypermethrin on semen quality and testosterone levels of rabbits. J Environ Sci Health B, 38,463–478.

Zhang SY, Ito Y, Yamanoshita O, Yanagiba Y, Kobayashi M, Taya K, Li C, Okamura A, Miyata M, Ueyama J, Lee CH, Kamijima M, Nakajima T. (2007). Permethrin may disrupt testosterone biosynthesis via mitochondrial membrane damage of Leydig cells in adult male mouse. Endocrinology, 148,3941–3949

Tables

Table 1. Effect of CDE on serum sexual hormones and protein profile in control and treated-groups

Groups		Parameters				
		Testosterone ng/ml	FSH ng/ml	LH ng/ml	Total protein g/dl	Albumin g/dl
Group I	Control	13.31±0.96	2.65±0.13	1.92±0.01	8.74±0.30	5.13±0.37
Group II	LCT	2.80±0.78 (-78.96%) a^{***}	3.78±0.14 (42.64%) a^{***}	2.93±0.09 (52.60%) a^{***}	4.96±0.12 (-43.25%) a^{***}	1.94±0.06 (-62.18%) a^{***}
Group III	CDE	13.49±0.65 (1.35%) b^{***}	2.46±0.05 (-7.17%) b^{***}	1.88±0.04 (-2.08%) b^{***}	8.58±0.28 (-1.83%) b^{***}	5.03±0.23 (-1.95%) b^{***}
Group IV	CDE+LCT	12.34±0.85 (-7.29%) b^{***}	2.90±0.11 (9.43%) b^{***} c^{*}	1.95±0.06 (1.56%) b^{***}	7.73±0.26 (-11.56%) b^{***}	4.05±0.08 (-21.05%) a^{**} b^{***} c^{*}

Data are expressed as means ± S.E. (n = 10 in each group).
a: Significant change with respect to control group; b: Significant change with respect to LCT-group; c: Significant change with respect to CDE-group.
*Significant change at $p<0.05$; **Highly significant change at $p<0.01$; ***Very highly significant change at $p<0.001$.
( ): % difference with respect to control value.

Table 2. Effect of CDE on serum lipid profile in control and treated-groups

Groups		Parameters				
		cholesterol mg/dl	TG mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl
Group I	Control	62.22±2.54	130.04±2.43	36.58±2.31	43.52±2.55	26.00±1.09
Group II	LCT	105.40±4.67 (69.40%) a***	213.01±3.56 (63.80%) a***	19.42±0.27 (-46.91%) a***	66.67±3.66 (53.19%) a***	42.60±3.11 (63.85%) a***
Group III	CDE	60.86±2.49 (-2.19%) b***	128.74±2.50 (-1.00%) b***	39.26±2.23 (7.33%) b***	42.95±3.55 (-1.31%) b***	25.75±1.10 (-0.96%) b***
Group IV	CDE+LCT	61.08±3.53 (-1.83%) b***	129.42±2.46 (-0.48%) b***	35.37±1.38 (-3.31%) b***	42.27±3.50 (-2.87%) b***	25.88±2.09 (-0.46%) b***

Data are expressed as means ± S.E. (n = 10 in each group).
a: Significant change with respect to control group; b: Significant change with respect to LCT-group; c: Significant change with respect to CDE-group.
*Significant change at $p<0.05$; **Highly significant change at $p<0.01$; ***Very highly significant change at $p<0.001$.
( ): % difference with respect to control value.

Table 3. Effect of CDE on cytokines,antioxidants and oxidative stress in control and treated-groups

Groups		Parameters					
		TNF-α pg/ml	IL-10 pg/ml	IL-12 pg/ml	GSH µg/g	SOD µg/g	MDA mµ/100g proteins
Group I	Control	44.10±3.69	117.92±4.63	83.78±4.42	12.97±0.41	19.36±0.54	0.64±0.03
Group II	LCT	144.22±6.63 (227.03%) a***	22.10±2.59 (-81.26%) a***	184.00±6.50 (119.62%) a***	6.90±0.29 (-46.80%) a***	9.09±0.60 (-53.04%) a***	1.25±0.02 (95.31%) a***
Group III	CDE	43.69±4.61 (-7.17%) b***	118.99±5.60 (0.91%) b***	82.50±4.71 (-1.53%) b***	13.07±0.31 (0.77%) b***	19.56±0.49 (1.03%) b***	0.65±0.01 (1.56%) b***
Group IV	CDE+LCT	62.16±4.92 (40.95%) b***	105.88±4.71 (-10.21%) b***	100.68±5.43 (20.17%) b***	10.99±0.20 (-15.27%) a** b*** c***	16.87±1.33 (-12.86%) b***	0.81±0.02 (26.56%) a*** b*** c***

Data are expressed as means ± S.E. (n = 10 in each group).
a: Significant change with respect to control group; b: Significant change with respect to LCT-group; c: Significant change with respect to CDE-group.
*Significant change at $p<0.05$; **Highly significant change at $p<0.01$; ***Very highly significant change at $p<0.001$.
( ): % difference with respect to control value.

Figures

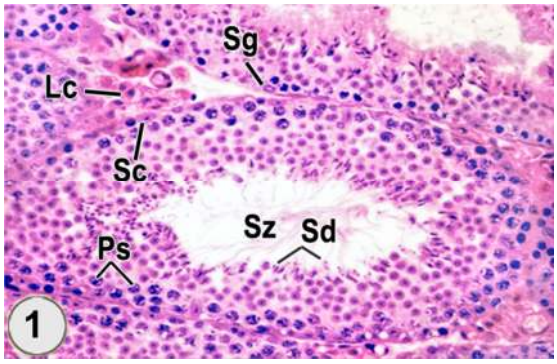

Figure 1.

Light micrograph of a testis section of control rats (group I) showing successive stages of spermatogenesis: the spermatogonia (Sg), primary spermatocytes (Ps), spermatids (Sd) and spermatozoa (Sz). The supporting Sertoli cells (Sc) are resting on the basal lamina. Aggregation of interstitial cells of Leydig (Lc) is also observed (H&E; 400x).

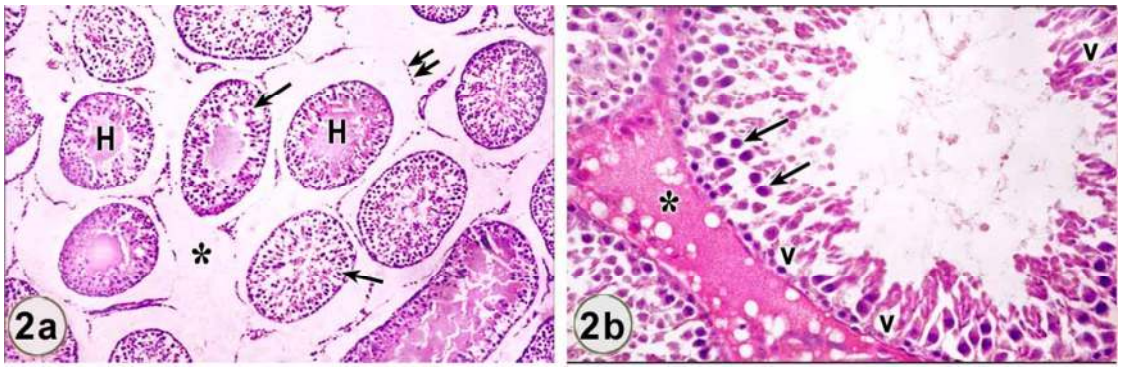

Figure 2.

Light micrographs of testis sections of LCT-treated rats (group II) showing **(a):** severe damage of the seminiferous tubules with separating of cells from underlying layer of the tubules (arrows), disappearance of many spermatogenic cells and hyalinization of the luminal contents (H). Note widening of intertubular spaces (*) and diminution of the stromal interstitial tissue (double arrows) (H&E; X100).**(b):** exfoliation (arrows) and depletion of germ cell with intraepithelial vacuoles (V). Notice interstitial edema (*) with a decrease in number of Leydig cells (H&E; 2a 100x & 2b 400x).

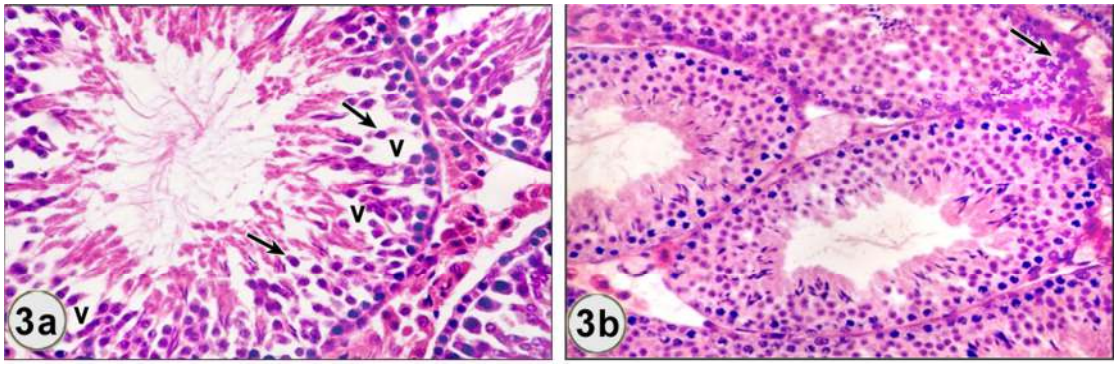

Figure 3.

Light micrographs of testis sections of rats treated with LCT plus CDE (group IV) showing **(a):** recovery of seminiferous tubules except separating of few cells from underlying layer (arrows) and appearance of intraepithelial vacuoles (V) (H&E; X400).**(b):** showing preservation of seminiferous epithelium with concentric layers of germinal lineage cells. However, few degenerated germ cells (arrows) are still seen (H&E; 3a &3b 400x).

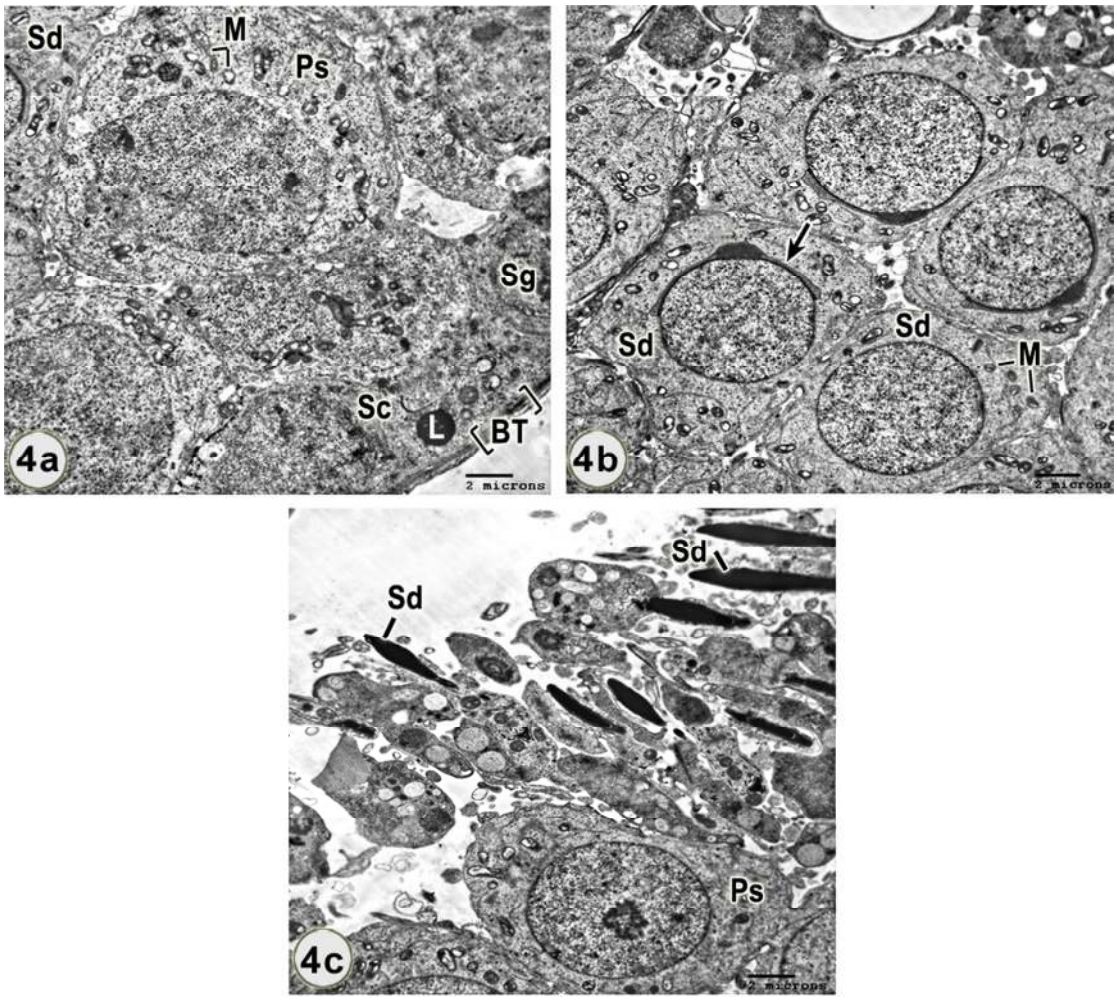

Figure 4.

Transmission electron micrographs of seminiferous tubules of control rats (group I) showing **(a):** boundary tissue (BT) and Sertoli cell (Sc) laying on the basal lamina in between the spermatogenic cells. Note the spermatogonium (Sg), primary spermatocytes (Ps) and spermatid (Sd).**(b):** a group of early differentiating spermatids (Sd) with the characteristic peripheral arrangement of mitochondria (M) and developing acrosomes (arrows).**(c):** heads of late elongating spermatids (Sd) contained strongly elongated, electron-dense nuclei covered anteriorly by acrosome caps. Notice presence of primary spermatocyte (Ps) (TEM, 4a-c 6000x).

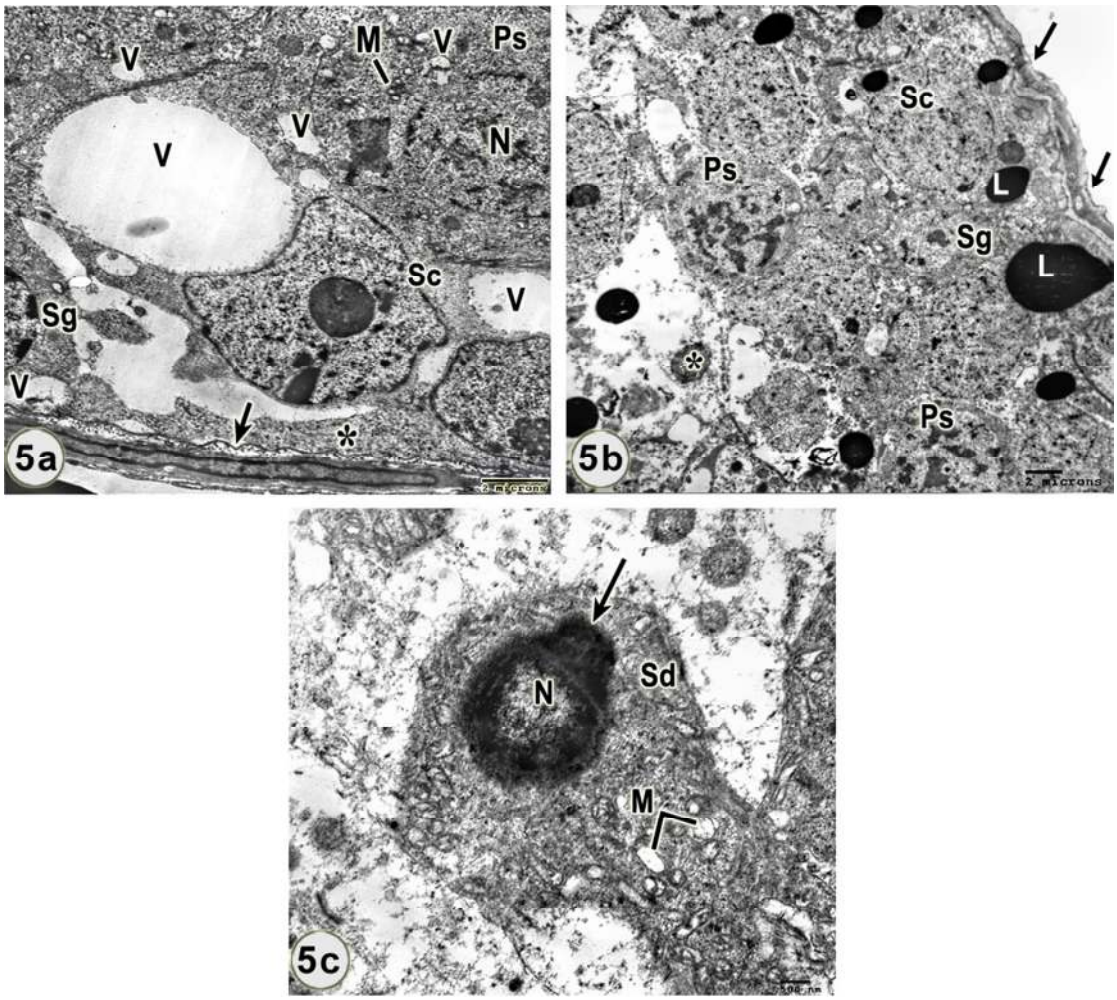

Figure 5.

Transmission electron micrographs of seminiferous tubules of LCT- treated rats (group II).**(a):** Note Sertoli cell (Sc) does not rest on the basement membrane but is separated from it by a part of cytoplasm (*). The cytoplasm of Sertoli cell, spermatogonia (Sg) and primary spermatocytes (Ps) is highly degenerated and show smooth endoplasmic reticulum-derived vacuoles (V). Primary spermatocyte show altered nucleus (N) and fused mitochondria (M).**(b):** Infolded boundary tissue (arrow) and numerous variable lipid globules (L) with marked edema in the disintegrated cytoplasm of Sertoli cells. The nuclear chromatin of some spermatogonia (Sg) and primary spermatocyte (Ps) is clumped and more electron dense with a reduction in the size of the cells. Abnormal cellular debris can also be seen in the tubular lumen (*).**(c):**Deformed spermatid (Sd) with an irregular outline, disorganized nucleus (N) with condensed chromatin and distorted acrosome. Note the degenerated mitochondria (M) (TEM, 5a8000x -5b 5000x- 5c 15000x).

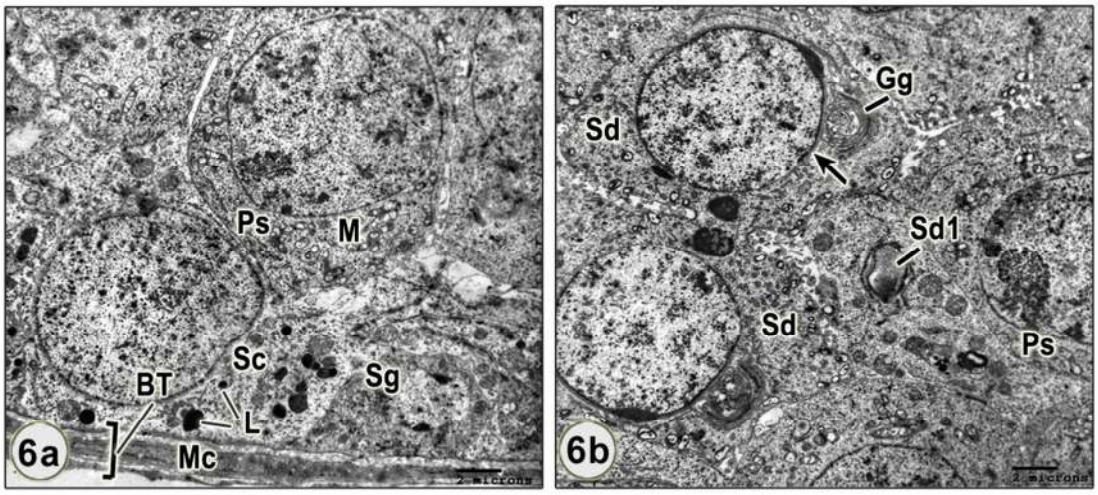

Figure 6.

Transmission electron micrographs of seminiferous tubules of rats treated with LCT plus CDE (group IV) showing **(a):** nearly normal structure of boundary tissue (BT) and relatively unaffected spermatogonium (Sg) and spermatocytes (Ps). Almost all the mitochondria appear normal except few fused ones (M). However, an increase in the number of lipid globule (L) of Sertoli cells is still observed. **(b):** two nearly normal spermatids (Sd) with their characteristic acrosomal vesicle (arrow); however, one deformed spermatid (Sd1) can be identified. Notice normal primary spermatocyte (Ps) with a large nucleus. MC, flat myoid cell; Gg, Golgi apparatus (TEM, 6a&b 6000x).