The Effect of "*Cleome droserifolia*" Extract Versus Stem Cells Therapy on The Changes Induced by ʎ-Cyhalothrin in Male Albino Rats

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Abstract

ʎ-cyhalothrin (LCT) is pyrethroid insecticide that is used worldwide for pest control in agriculture and household use. *Cleome droserifolia* extract (CDE) is a potential antioxidant protecting cells from oxidative stress. Mesenchymal stem cells (MSCs) have the capacity to generate multiple distinct cell lineages. The present study investigates the protective and therapeutic effect of CDE and MSCs, separately on LCT-induced changes in male rats. Seventy adult male albino rats were divided into seven groups: **group I:** served as control; **group II:** received LCT i.p. only (6.2 mg/kg b.wt.); **group III:** received CDE only (100 mg/kg b.wt., p.o.) for eight weeks; **group IV:** received CDE as a protective agent daily for eight weeks, then followed by the administration of LCT (i.p.) three times a week for two weeks; **group V:** exposed to LCT (i.p.) three times a week for two weeks, then treated with the CDE daily for 8 weeks; **group VI:** rats injected (i.v.) with a single dose of MSCs ($1x10^6$ cells/cm^2 saline), then received dose of LCT (i.p.) three times a week for two weeks and **group VII:** rats received dose of LCT (i.p.) three times a week for two weeks, then injected (i.v.) with a single dose of MSCs. Results showed that, LCT caused significant reduction in the body and testis weight, and markedly impaired sperm quality (a count, viability, motility and abnormality). Also, LCT elicited significant declines in serum testosterone, whereas, serum follicle stimulating hormone (FSH) and luteinizing hormone (LH) were significantly increased. Compared to LCT-treated animals, CDE in the protective group markedly restored the alteration of body and testis weights, sperm indices and sexual hormones. However, CDE in the curative group was found to be less effective in restoring LCT induced morphological and biochemical alterations. Transplantation of MSCs as being a therapy after LCT intoxication showed significant improvement in the activity of all parameters rather than their protective effect which showed minimal protection against LCT. In conclusion, data of this study revealed that the protective by CDE and therapeutic by MSCs are more effective than therapeutic by CDE and protective by MSCs in ameliorating LCT-induced testicular damage and improvement of male fertility and reproductive functions.

Key Words: Cleome droserifolia, Mesenchymal stem cells, ʎ-cyhalothrin, Sexual hormones, Rat.

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1.Introduction

ʎ-cyhalothrin (LCT), a new generation type II synthetic pyrethroid insecticide, has extensive uses as an agro-pesticide ***(Fetoui et al., 2009)***. It is widely used in Egypt and valued for its broad-spectrum control on a wide range of pests in a variety of applications ***(Abdel Aziz & 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 (LPO) 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, family Cleomaceae, commonly grown in different areas of North Sinai, Egypt. *Cleome* species are generally used in folk medicine as stomachics, rubefacients and in the treatment of scabies, rheumatic fever and inflammation ***(EL-Shenaway et al., 2006)***. The dried herb of *C. droserifolia*, locally 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 for *C. droserifolia*is rich in bioactive compounds as flavonoids, flavonol glycosides, alkaloids, tannins and steroids ***(Nagy and Mohamed, 2014)***. Flavonoids from *C. droserifolia* 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 a major role in treating or retarding a wide spectrum of diseases and reported to possess anti-oxidative, antiatherosclerotic, anti-inflammatory, antitumor, antithrombogenic, antiosteoporotic, and antiviral properties ***(Nijveldt et al., 2001)***. Antioxidants protect deoxyribonucleic acid (DNA) and other important molecules from oxidation and damage, and can improve sperm quality and consequently increase fertility rate in men ***(Yang et al., 2006)***.

Stem cells have recently generated more public and professional interest than almost any other topic in biology. One reason stem cells capture the imagination of so many is the promise that understanding their unique properties may provide deep insights into the biology of cells as well as a path toward treatments for a variety of degenerative illnesses ***(Fouda, 2011)***. The importance of the bone marrow (BM) microenvironment in the maintenance and regulation of hematopoietic stem cells has been reviewed widely ***(Dorshkind, 1990 and Deryugina & Muller-Sieburg, 1993)***. Mesenchymal stem cells (MSCs) reside in the BM and give rise to multiple mesodermal tissue types, including bone, cartilage, tendon, muscle, fat and marrow stroma ***(Beresford, 1989 and MacMillan et al., 2009)***.

The present study aimed to comparison between the protective and therapeutic effects of a natural antioxidant, *Cleome droserifolia* extract (CDE) and mesenchymal stem cells (MSCs) against toxic effects of ʎ-cyhalothrin (LTC) on male rats.

2.Materials and methods

2.1.Animals

Seventy adult male Wistar albino rats, their average body weight were 120±10 g/animal representing 2-3 months of age, were used in the present study. The experimental animals 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 Ethics Committee for research on laboratory animals at Cairo University.

2.2.ʎ-cyhalothrin

ʎ-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 intraperitoneally (i.p.) administrated at a dose of 1/10 LD_{50} (6.2 mg/kg/b.w.) ***(Fetoui et al., 2013)***.

2.3.Natural antioxidant (Cleome droserifolia)

The raw material was collected from Arish, North Sinai, Egypt, and authenticated to *C. droserifolia* by Agricultural Researches Center, Giza, Egypt. The *C. droserifolia* herb was spread over the bench and left for drying in the shade, then reduced to a powder. Decoction of the plant material was prepared by boiling 400 g of the dry plant material in 6 liters of tap water for 2 minutes and then filtered after 10 minutes. To minimize the volume of the decoction, it was concentrated in 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 on the day of experimental studies and administered by gavages at 100 mg/kg/b.w. ***(El-Naggar et al., 2005)***.

2.4.Stem cells

Bone marrow derived stem cells, one important source of mesenchymal stem cells (MSCs), have been isolated and cultured in Medical Research Center, Kasr Al-ainy Medicine Faculty, Cairo University.

Ten adult male albino rats with average weight (200-300g) were used to prepare the MSCs by the modified method from ***Soleimani & Nadri (2009)***. MSCs were marked using 50 micron iron oxide in 4 ml RPMI media for 30 min followed by centrifugation at 2000xg for 10 min. MSCs will be verified by morphology ***(Rastegar et al., 2010)***.

The MSCs are spindle- shape cells; The International Society of Cryotherapy has devised three criteria needed to identify MSCs : a) Plastic adherence of the cells isolated in culture. b) Expression of cluster of differentiation (CD) markers such as CD105, CD73, and CD90 in > 95% of the culture with absent expression of markers including CD34, CD45, CD14 or CD11B, CD79A or CD19 and human leukocyte antigen-DR (HLA-DR) in > 95% of the culture.

2.5.Experimental design

Rats were divided into seven groups (each 10 animals) as follows: **Group I:** control, which received distilled water only throughout the experiment (p.o.) daily; **Group II:** LCT group, which was given ʎ-cyhalothrin only (6.2 mg/kg b.w.,i.p.) three times a week for two

weeks; **Group III:** CDE group, in which the animals received *Cleome droserifolia* extract only (100 mg/kg b.w.,p.o.) in distilled water daily for eight weeks; **Group IV (Protective by CDE):** animals were given CDE (p.o.) daily for eight weeks. On the 7^{th} week they received LCT (i.p.) three times a week for two weeks; **GV (Therapeutic by CDE):** Rats receiving dose of LCT (i.p.) three times a week on the 1^{th} and 2^{th} weeks, then administered dose of CDE (p.o.) daily for 8 weeks. **GVI (Protective by MSCs):** Rats injected (i.v.) with MSCs through the tail vein with a single dose ($1x10^6$ cells/cm^2 saline/animal), then received dose of LCT (i.p.) three times a week for two weeks, and left until the 8^{th} week. **GVII (Therapeutic by MSCs):** Rats received dose of LCT (i.p.) three times a week on the 1^{th} and 2^{th} weeks, then injected (i.v.) with MSCs and left for 8 weeks.

At the end of the experiment period, blood samples were collected through orbital sinus, in a clean centrifuge tube. Blood was centrifuged at 3000 rpm for 15 min and serum was collected for biochemical analysis then, all animals were sacrificed and the testes were immediately excised.

2.6.Determination of total body and testis weight

Animals of control and treated groups were weighed prior to the time of treatment and again prior to sacrifice. Also, the testes were rapidly removed, blotted with a piece of filter paper and weighed.

2.7.Sperm indices

After sacrificing rats, the caudal epididymis was dissected out and placed in 2 ml of 0.9% physiological saline. It was cut into small pieces to release the mature sperms in solution ***(Blandau & Jordan, 1941)***. Concentration of sperm cells was determined using the haematocytometer according to the technique adopted by ***Bearden & Fuquay (1980)***. Using eosin as a differential stain for staining dead sperm cells (it can't pass through living cell membrane) and nigrosin as a background stain, the percentage of live sperm was determined ***(Blom, 1950)*** . Sperm motility was examined according to the method reported by ***Bearden & Fuquay (1980)***. A small drop of the cell suspension was put on the slide and the spread slides were air dried without fixation for about 24 hours. Slides were stained with haematoxylin and eosin. Three slides were prepared for each rat ***(Wyrobek & Bruce, 1978)***. Sperm smears were examined by light microscopy. For each rat 500 sperms were examined and morphological abnormalities were recorded according to the criteria of ***El- Nahas et al. (1989)***.

2.8.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 & Behrman (1974)***. Follicle stimulating hormone (FSH) and luteinizing hormone (LH) were estimated depending on the assays depicted by ***Santner et al. (1981)*** and ***Nankin (1972)***, respectively.

2.9.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 & Crewson, 2003)***.

3.1 Results

3.1.1Detecting of mesenchymal stem cells (MSCs)

In vitro, the cell culture showed mesenchymal stem cells (MSCs) as spindle and fibroblastic in morphology which later became the rounded cells (Fig.1). MSCs groups showed spindle-shaped, branched, and globular-shaped Prussian blue positive-stained cells in the interstitial space of the seminiferous tubule (Fig.2).

3.1.2.Effect of CDE and MSCs on LCT-induced alteration in total body weight

From the present investigation, Table (1) cleared that the data concerned with the control rats (GI) showed an increase in body weights. The average body weight at the beginning of the experiment was 120±10 g/animal and reached (149.88±1.32) at the end of experiment. In contrast, there was a very highly significant decrease in the mean value of LCT rats (GII) that recorded (90.52±4.30) compared to control group. Furthermore, protective and therapeutic groups by CDE (GIV and GV) showed significant increase ($p<0.001$) in total body weight, the mean values recording (144.94±2.39 and 131.81±3.33), respectively as compared to the LCT group. Also, the mean values of body weight recorded significant increased in the protective group (122.03±3.41) and therapeutic group (134.27±2.33) with MSCs when compared to LCT rats (90.52±4.30).

3.1.3.Effect of CDE and MSCs on LCT-induced alteration in testis weight

The data represented in Table (1) revealed a very highly significant decrease in total testis weight of LCT group (GII) recorded a change (-33.96%) as compared to control group. A very highly significant increase occurred in the mean testis weight in both, protective group by CDE (GIV) and therapeutic group by MSCs (VII) as compared to LCT group. The percentage of the mean testis weight reached (43.81% and 31.43%), respectively. In addition, the average of testis weights of therapeutic group with CDE (GV) recorded highly significant increase and reached (29.52%) compared to LCT group. While, a non-significant increase occurred in the mean testis weight in protective rats by MSCs (GVI) as compared to LCT group. The percentage reached (9.52%).

3.1.4.Effect of CDE and MSCs on LCT-induced alteration in sperm indices

3.1.4.1.Sperm count:

The results in Table (2) recorded a very highly significant decrease in sperm count of LCT rats (GII) reached (-62.13%) in comparison with control (GI). In the protective rats treated with CDE (GIV), the value was nearly returned to the control and reached (-6.36%). whereas, the sperm count was showed partial improvement in (GV, GVI and GVII), which recorded (-20.21%, -37.10% and -24.19%), respectively as compared to control group.

3.1.4.2.Sperm viability:

Data recorded for the sperm viability were presented by Table (2). The sperm viability of LCT group (GII) was very highly significant decrease compared to the control group, the percentage of change were (-34.58%). Furthermore, it is clear that there were statistical significant ($p<0.001$) improvement of the sperm viability percentage in (GIV, GV and GVII), with a mean value of (84.81±3.09, 81.68±3.13 and 81.87±3.14), respectively compared to (61.83±2.01) for LCT group (GII), whereas, the protective by MSCs caused partial recovery in the percentage of sperm viability (71.89±2.06) in (GVI) as compared to LCT group.

3.1.4.3.Sperm motility:

The results obtained in Table (2) recorded a very highly significant ($p<0.001$) decrease in the sperm motility level in rats administered by LCT (GII) and recorded (37.48±5.31) compared to control (95.31±3.14), with a percent of change (-60.68%). After protective with CDE (GIV), the sperm motility level was nearly similar to that in control group and reached (80.49±4.41), with a percent of change (-15.55%) and (114.75%) in relation to (GI) and (GII), respectively. In therapeutic rats by both CDE and MSCs (GV and GVII), a significant ($p<0.01$) elevation in the sperm motility level as compared to LCT rats, were recorded (68.74±6.37 and 70.80±2.31), respectively. The percentage of increase was (83.40% and 88.90%) as compared to LCT group. While, the average value of protective by MSCs (GVI) was (58.90±4.43), with a percent of change (57.15%) in relation to LCT group.

3.1.4.4.Sperm abnormality:

Table (2) showed the sperm abnormality level in both normal and experimental groups . A very highly significant increase ($p<0.001$) in the sperm abnormality level was observed in the LCT group (36.20±3.28) compared to control group (8.83±1.08), the percentage of change was (309.97%). In relation to the control group, (GIV, GV and GVII) showed a great reduction in the percentages of change of sperms deformation. The percentages of change were (28.09%, 71.91% and 53.34%), respectively. While, very slight decrease occurred in the sperm abnormality of protective by MSCs (GVI). The percentage increment was (127.63%) as compared with the control group.

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

3.2.1.Testosterone hormone:

Data recorded for the levels of serum testosterone hormone were presented by Table (3) and Fig.(3). Present results revealed that the serum testosterone level recorded very highly significant decrease ($p<0.001$) in LCT rats (GII) was found to be (2.80±0.78) as compared to control (13.31±0.96), with a percent of change (-78.96%). The level of testosterone revealed a non-significant decrease ($p>0.05$) in protective with CDE (GIV) compared to control groups, with average value of (12.34±0.85) and percent of change (-7.29%). Furthermore, the therapeutic groups by both CDE (GV) and MSCs (GVII) showed statistical increase ($p<0.001$) as compared to LCT group. The percentage of change for (GVII) in relation to control groups decreased than that of (GV), where the percent of change for (GVII) were (-27.05%) compared to control, while the percentage of change for (GV) were (-41.47%) compared to control group. By another way, the data obtained from protective group by MSCs showed very slight improvement in testosterone level compared to the control group, with percentage of change (-55.60%).

3.5.2.Follicle stimulating hormone (FSH):

From the inspection of the data presented in Table (3) and Fig.(4), the FSH level of LCT rats (GII) showed significant elevation ($P<0.001$) reaching to 42.64% compared with control group. Protective group by CDE (GIV) and therapeutic group by MSCs (GVII) induced very highly significant improvement of serum FSH level reaching (9.43% and 15.09%), respectively in comparison to control and (-23.28% and -19.31%), respectively in comparison to LCT group. On the other hand, the FSH level of therapeutic group by CDE (GV) and Protective group by MSCs (GVI) revealed minimal improvement, especially (GVI)

as compared to control, with a percent of change (25.28%) for (GV) and (39.62%) for (GVI) compared to the control group.

3.5.3.Luteinizing hormone (LH):

On detecting the serum LH level, the data are given in Table (3) and Fig.(4). In relation to the control rats, a very highly significant increase ($p<0.001$) was obtained in serum LH level in LCT administered group (GII) recorded the mean value (2.93±0.09) compared to control group (1.92±0.01). Moreover, the data recorded that the best improvement occurred in the protected group by CDE (GIV) in relation to LCT rats reaching (1.95±0.06), with a percent of change (-33.45%). Furthermore, the LH level was recovered in both groups, therapeutic by CDE (GV) and therapeutic by MSCs (GVII), where LH level reached (2.35±0.09) for (GV) and (2.23±0.01) for (GVII) with a percent of change (-19.80%) and (-23.89%), respectively in relation to LCT group. On the other hand, there was not any improvement observed in protective group by MSCs (GVI) of LH level, the mean was (3.25±0.02), with a percent of change (10.92%) compared to LCT group.

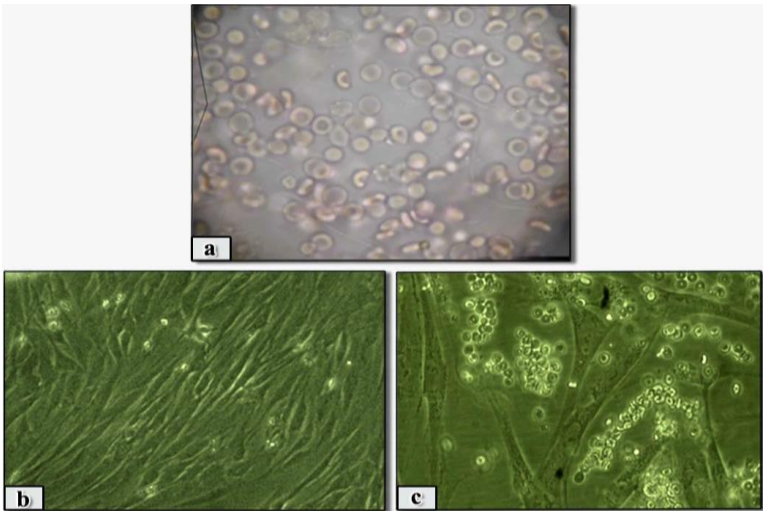

Figure (1): Photomicrographs of morphological changes of MSCs in tissue cultured.

(a): Culture containing nonadherant cells isolated by enzymatic method from human adipose tissue. The cells are round with large nuclei surrounded by cytoplasm, 1 day after isolation, later change morphological changes seen (X100).

(b): image of 5-day-old nonpassaged MSCs with typical fibroblast-like cells with fusiform shape (X100).

(c): Proliferation of MSCs, rounded daughter cells are released into the suspension. Contrast-phase inverted microscope (X400).

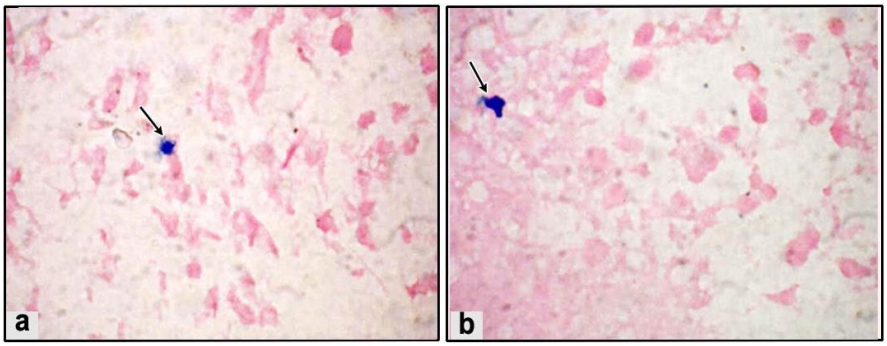

Figure (2): Photomicrograph of testis sections of a stem cell treated rats (GVI and GVII) showing a spindle, branched and globular-shaped prussian blue positive-stained cell (Iron-tagged MSC) homing in the interstitial space (a & b) of testicular tissue (arrow), (40X).

Table 1. The protective and therapeutic role of CDE and MSCs on total body and testis weights in treated groups with LCT.

Groups		Parameters	
		Total body weights (g)	***Testis weight (g)***
Group I	***Control***	149.88±1.32	1.59±0.03
Group II	***LCT***	90.52±4.30 (-39.61%) a^{***}	1.05±0.09 (-33.96%) a^{***}
Group III	***CDE***	155.74±2.33 (3.91%) b^{***}	1.73±0.02 (8.81%) b^{***}
Group IV	***Protection by CDE***	144.94±2.39 (-3.30%) b^{***}	1.51±0.04 (-5.03%) b^{***}
Group V	***Therapy with CDE***	131.81±3.33 (-12.72%) a^{***} b^{***} c^{***} d^{*}	1.36±0.07 (-14.47%) a^{*} b^{**} c^{***}
Group VI	***Protection by MSCs***	122.03±3.41 (-18.58%) a^{***} b^{***} c^{***} d^{***}	1.15±0.04 (-27.67%) a^{***} c^{***} d^{***}
Group VII	***Therapy with MSCs***	134.27±2.33 (-10.41%) a^{**} b^{***} c^{***}	1.38±0.03 (-13.21%) b^{***} c^{***} f^{*}

Data are expressed as means ± S.E. (n=10 in each group).
a=compared to control group (GI); b=compared to GII; c=compared to GIII; d=compared to GIV; e= compared to GV; f=compared to GVI.
*=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. The protective and therapeutic role of CDE and MSCs on sperm count, viability, motility and abnormality in treated groups with LCT.

Groups		Parameters			
		Sperm count ($x10^6$/ml)	***Sperm viability (%)***	***Sperm motility (%)***	***Sperm abnormality (%)***
Group I	***Control***	195.98±3.06	94.51±3.20	95.31±3.14	8.83±1.08
Group II	***LCT***	74.21±4.89 (-62.13%) a^{***}	61.83±2.01 (-34.58%) a^{***}	37.48±5.31 (-60.68%) a^{***}	36.20±3.28 (309.97%) a^{***}
Group III	***CDE***	194.40±2.16 (-0.81%) b^{***}	95.62±3.21 (1.17%) b^{***}	96.57±3.21 (1.32%) b^{***}	8.69±1.04 (-1.59%) b^{***}
Group IV	***Protection by CDE***	183.52±3.08 (-6.36%) b^{***}	84.81±3.09 (-10.26%) b^{***}	80.49±4.41 (-15.55%) b^{***}	11.31±2.22 (28.09%) b^{***}
Group V	***Therapy with CDE***	156.37±5.07 (-20.21%) $a^{***}b^{***}c^{***}d^{***}$	81.68±3.13 (-13.58%) $a^{*}b^{***}c^{*}$	68.74±6.37 (-27.88%) $a^{**}b^{***}c^{***}$	15.18±2.30 (71.91%) b^{***}
Group VI	***Protection by MSCs***	123.27±4.67 (-37.10%) $a^{***}b^{***}c^{***}d^{***}e^{***}$	71.89±2.06 (-23.93%) $a^{***}c^{***}d^{*}$	58.90±4.43 (-38.20%) $a^{***}b^{*}c^{***}d^{*}$	20.10±2.20 (127.63%) $a^{**}b^{***}c^{**}$
Group VII	***Therapy with MSCs***	148.57±3.88 (-24.19%) $a^{***}b^{***}c^{***}d^{***}f^{***}$	81.87±3.14 (-13.37%) $a^{*}b^{***}c^{*}$	70.80±2.31 (-25.72%) $a^{**}b^{***}c^{**}$	13.54±1.19 (53.34%) b^{***}

Data are expressed as means ± S.E. (n=10 in each group).
a=compared to control group (GI); b=compared to GII; c=compared to GIII; d=compared to GIV; e= compared to GV; f=compared to GVI.
*=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. The protective and therapeutic role of CDE and MSCs on sexual hormones in treated groups with LCT.

Groups		Parameters		
		testosterone (ng/ml)	***FSH (ng/ml)***	***LH (ng/ml)***
Group I	***Control***	13.31±0.96	2.65±0.13	1.92±0.01
Group II	***LCT***	2.80±0.78 (-78.96%) a^{***}	3.78±0.14 (42.64%) a^{***}	2.93±0.09 (52.60%) 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^{***}
Group IV	***Protection by CDE***	12.34±0.85 (-7.29%) b^{***}	2.90±0.11 (9.43%) $b^{***}c^{*}$	1.95±0.06 (1.56%) b^{***}
Group V	***Therapy with CDE***	7.79±0.90 (-41.47%) $a^{***}b^{***}c^{***}d^{**}$	3.32±0.07 (25.28%) $a^{***}b^{*}c^{***}d^{*}$	2.35±0.09 (22.40%) $a^{***}b^{***}c^{***}d^{***}$
Group VI	***Protection by MSCs***	5.91±0.76 (-55.60%) $a^{***}c^{***}d^{***}$	3.70±0.06 (39.62%) $a^{***}c^{***}d^{***}$	3.25±0.02 (69.27%) $a^{***}b^{**}c^{***}d^{***}e^{***}$
Group VII	***Therapy with MSCs***	9.71±0.59 (-27.05%) $a^{*}b^{***}c^{*}f^{*}$	3.05±0.05 (15.09%) $b^{***}c^{***}f^{***}$	2.23±0.01 (16.15%) $a^{**}b^{***}c^{***}d^{*}f^{***}$

Data are expressed as means ± S.E. (n=10 in each group).
a=compared to control group (GI); b=compared to GII; c=compared to GIII; d=compared to GIV; e= compared to GV; f=compared to GVI.
*=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.

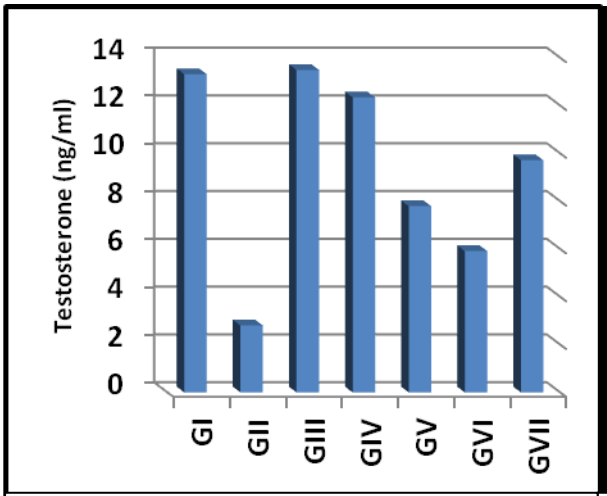

Figure (3): The protective and therapeutic role of CDE and MSCs on testosterone in treated groups with LCT.

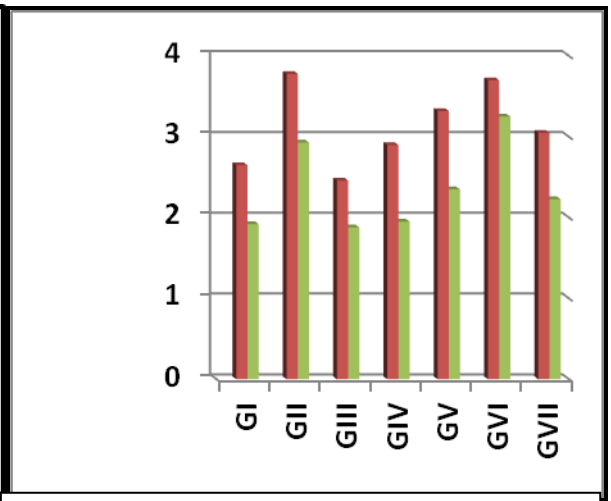

Figure (4): The protective and therapeutic role of CDE and MSCs on follicle stimulating hormone (FSH) and luteinizing hormone (LH) in treated groups with LCT.

3.2 Discussion

The present study showed that, there was significant decrease in the total body and testis weight in group of rats treated with LCT only (GII) as compared to control (GI). The decrease in body weight in male rats treated with LCT is probably attributed to the loss of appetite and/or a consequence of disturbance in metabolic processes due to marked damage in the liver tissue. This suggestion is in accordance with ***Madbouly (2003)*** and ***Prashanthi et al. (2006)*** who stated that an insecticide induced a marked damage in the liver tissue and affected the metabolic processes in the liver and that effect was due to diminished food and water intake in treated mice DZN group. In addition, ***Friedmann (2002)*** concluded that the effects of atrazine on the reproductive tract may not be direct but rather, the noted deficits of the male reproductive tract resulted from reduced food intake by the treated animals.

In this study LCT caused a significant reduction in the testis weight which might be due to the decrease in serum testosterone levels. These results are in agreement with the findings by ***Anderson et al. (2002)*** who mentioned that the weight of testis is basically dependent on the mass of the differentiated spermatogenic cells, thus the reduction in the weight of the testis may be due to decreased number of germ cells, inhibition of spermatogenesis and steroidogenic enzyme activity ***(Hatjian et al., 2000 and Fattahi et al., 2009)***. Also, may be due to decreased acetylcholinesterase level and spermatogenic cells, Sertoli cells and Leydig cells losses and testicular atrophy ***(Dutta & Meijer, 2003)***.

The protective and therapeutic groups by CDE (GIV and GV) showed significant increase in total body weight as compared to the LCT group (GII). Similarly, both absolute and relative testis weights manifested significant increase in protective group (GIV) while there was partial increase in the therapeutic group (GV). Similar results were demonstrated by ***El-Shenawy & Abdel-Nabi (2004)*** who revealed strong amelioration in both body and testis weights in diabetic rats treated with CDE. This confirms that the CDE reduce the generation of oxygen free radicals in the body, resulting in lesser oxidative damage to the tissues, and enhanced levels of antioxidant potential activities in the LCT rats. In accordance with previous literature ***Kumar et al. (2009)*** and ***EL-Khawaga et al. (2010)*** the reason attributed to the existence of several flavonoids in CDE.

The present study showed a partial increase of total body weight in protective group by MSCs (GVI) as compared to LCT group. Similarly, testis weight manifested partial increase in the same group. While, there was significant increase in total body weight and testis weight in the therapeutic by MSCs as compared to LCT group. Then from the present data it may be expected that, MSCs could migrate and home to the various injured organs, make repairing through MSCs mechanisms and trans-differentiate into specific cells and thus increased weight gradually. This suggestion is in agreement with several results by ***Zhao et al. (2002) and Meirelles et al. (2008)*** who reported that stem cells can regenerate various cell lineages by trans-differentiation or cell fusion mechanisms. It has been proven that MSCs that isolated from bone marrow are able to differentiate into different mesodermal cell lineages including bone, cartilage, muscle, fat and other connective tissues cells ***(Caplan, 2007)***. Moreover, MSCs in tissue repair would accelerate healing because they are trophic and immune-modulatory, and they can be directly delivered to damaged areas in large numbers or home to injured sites after systemic infusion due to the expression of specific extracellular matrix and chemokine receptors ***(Meirelles et al., 2008)***. Stem cells may also act by up-regulating the anti-apoptotic protein Bcl-2 and suppressing apoptosis ***(Chen et al., 2001)***, these processes are thought to contribute to the regeneration of normal cells in the damaged organ ***(Pai et al., 2012)***.

This study revealed that rats treated with LCT (GII) had markedly impaired sperm quality. LCT significantly lowered sperm count, viability and motility percent and significantly increased sperm abnormalities. These findings may be due to an adverse effect of LCT on spermatogenesis by the affecting on testosterone secretion which essential to maintain the structure and function of the male accessory sex gland, thus a lack of testosterone disrupts spermatogenesis. Results of the present study are in accordance with the findings of ***Nada et al., 2010 and Oda & El-Maddawy (2011)***.

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 ***(Oda & El-Maddawy, 2011)***. 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)***, so reduction in sperm quality subsequently reduction in fertility according to the regardations of ***Agnes & Akborsha (2003)***.

The administration of CDE in the present investigation (GIV and GV) indicated significant improvement as regards sperm count, viability, motility and sperm abnormality especially in protective rats group (GIV) in relation to LCT rats (GII). It has been postulated that vitamin C which has proved its presence in the *Cleome* species minimizes testicular cytotoxic effects through prevention the production of the mutagenic electrophilic metabolites and stimulation of 7-X-hydroxylation of lipids and cholesterol nuclei, thus enhancing their degradation to bile acids which could be excreted from the body, such antioxidant action of vitamin C could relieve the germ cells from oxidative damage thereby decreasing the percentage of abnormal sperms. These findings are in agreement with the results observed by ***Usha et al. (2003)*** and ***Oladele & Abatan (2010)***. Also, ***Levine (1986)*** implicated the role of ascorbic acid (vitamin C) in the physiology of testis in regard to protein metabolism. Many enzymatic functions of ascorbic acid are believed to be essential for the normal integrity and function of testis i.e. synthesis, development and maintenance of normal sperm ***(Dawson et al., 1990)***, thus regulate protein metabolism and repair activities in the germinal cells.

In the present experiment, the protective group by MSCs (GVI) showed partial improvement in sperm count, viability, motility and sperm abnormality percent. But, significant improvement was observed in therapeutic group by MSCs (GVII) in the same parameters as compared to LCT group. This improvement might be due to that MSCs could differentiate into germ cells. These findings are in agreement with the results observed by ***Hassan & Alam (2014)*** and ***Abd El-Dayem et al. (2015)***. MSCs were the candidates for germ cell differentiation *in vivo* and *in vitro* ***(Zhu et al., 2012)***. Another cytoprotective mechanism of MSCs may involve the paracrine effects by secretion of growth factors ***(Wang et al., 2009)***.

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 (GII). This may be due to disruption of the feedback mechanisms existing between hypothalamic-pituitary-gonadal axes, decrease 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 & El-Maddawy (2011)***. 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 into mitochondria ***(Miller, 2007)***. Testosterone synthetic enzymes, primarily cytochrome P450scc, $P450_{17\alpha}$and 17beta hydroxysteroid dehydrogenase (17β-HSD), play a critical role in testosterone synthesis in Leydig cells. P450scc initiates the first enzymatic step in testosterone biosynthesis in the inner mitochondrial membrane of Leydig cells, where cholesterol is

converted to pregnenolone. Pregnenolone is catalyzed by $P450_{17\alpha}$ to produce 17-hydroxyprogesterone and androstenedione ***(Wang et al., 2010)***.

The testosterone, FSH and LH hormones were improved significantly in protective rats group with CDE (GIV), and these effects are supported by ***Helal et al. (2002)***. The improvement of hormone levels may be attributed partially to the amelioration in the production of pituitary gonadotropins, high leptinemia and low neuropeptide Y production ***(Himms-Haagen, 1999)***; is a regulator of gonadotropin releasing hormone production which induces the production of pituitary gonadotropins ***(Keisler et al., 1999)***. Moreover, quercetin and rutin, the major active antioxidant flavonoids in CDE, stimulated steroid hormone synthesis in MA-10 cells through up-regulation of steroidogenic acute regulatory promoter activity and mRNA expression and 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 ***(Chen et al., 2007)***.

The present investigation showed that MSCs in the protective group (GVI) revealed minimal improvement in sexual hormones (testosterone, FSH and LH) levels. While, in therapeutic group by MSCs (GVII) showed significant improvement in the levels of these hormones compared with LCT group. These findings are in agreement with the results observed by ***Hassan & Alam (2014)*** and ***Abd El-Dayem et al. (2015)***. This improvement might be due to capacity of MSCs to differentiate into steroidogenic cells, both in vivo and in vitro ***(Herrera et al., 2007 and Monsefi et al., 2013)*** also, MSCs represent not only a powerful tool for studies of the differentiation of the steroidogenic lineage but may also offer a possible clinical stem cell resource for diseases of steroidogenic organs. In addition, another study has demonstrated that adult stem cells derived from bone marrow can differentiated into Leydig and Sertoli cells in rat testis ***(Yazawa et al., 2006 and Lue et al., 2007)***.

4.Conclusion

The results of this study indicated that the protective effect of CDE and therapeutic effect of MSCs were more effective than therapeutic and protective effects for CDE and MSCs, respectively in ameliorating LCT-induced testicular damage and for improvement of male fertility and reproductive functions.

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الملخص باللغة العربية

تأثير العلاج بمستخلص السموه والخلايا الجذعية على التغيرات التي يحدثها مبيد λ- سيهالوثرين في ذكور الجرذان البيضاء

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3-قسم علم الحيوان- كلية العلوم- جامعة عمر المختار- ليبيا.

تهدف هذه الدراسة إلى تقييم الدور الوقائي والعلاجي المحتمل لكل من مستخلص نبات السموه *(Cleome droserifolia)* والخلايا الجذعية الميزنشيمية كلاً على حده في القدرة على استعادة الوظائف التناسلية في ذكور الجرذان وذلك بعد إحداث اضطراب في وظائف الخصية من خلال التعرض لمبيد λ-سيهالوثرين (λ-cyhalothrin).

وقد استخدم في هذه الدراسة عدد 70 من ذكور الجرذان البالغة ، قسمت إلى 7 مجاميع ، **المجموعة الأولى:** وهي المجموعة الضابطة ، وفيها أعطيت الجرذان ماء مقطر فقط عن طريق الفم لمدة 8 أسابيع يومياً ، **المجموعة الثانية:** حقنت بريتونياً بمبيد λ-سيهالوثرين (6.2 مجم/كجم من وزن الجسم) ثلاث مرات أسبوعياً لمدة أسبوعين ، **المجموعة الثالثة:** أعطيت مستخلص نبات السموه منفرداً (100 مجم/كجم من وزن الجسم) يومياً عن طريق الفم لمدة 8 أسابيع ، **المجموعة الرابعة:** أعطيت مستخلص النبات عن طريق الفم كعامل وقائي يومياً لمدة 8 أسابيع ثم حقنت بريتونياً بالمبيد بدءاً من الأسبوع السابع ثلاث مرات أسبوعياً لمدة أسبوعين ، **المجموعة الخامسة:** حقنت بريتونياً بالمبيد ثلاث مرات أسبوعياً عند الأسبوعين الأول والثاني ثم أعطيت مستخلص النبات كعلاج يومي عن طريق الفم لمدة 8 أسابيع ، **المجموعة السادسة:** حقنت وريدياً بجرعة واحدة من الخلايا الجذعية (مليون خلية في ملح الفوسفات المنظم) كوقاية ثم حقنت بريتونياً بالمبيد ثلاث مرات أسبوعياً لمدة أسبوعين وتركت حتى الأسبوع الثامن ، **المجموعة السابعة:** حقنت بريتونياً بالمبيد ثلاث مرات أسبوعياً عند الأسبوعين الأول والثاني ثم حقنت وريدياً بجرعة واحدة من الخلايا الجذعية كجرعة علاجية وتركت لمدة 8 أسابيع.

تشير نتائج هذه الدراسة إلى أن مبيد λ-سيهالوثرين قد أحدث نقصاً ذا دلالة احصائية في أوزان الجسم والخصى ، واختلال ملحوظ في كفاءة الحيوانات المنوية. وعلى المستوى البيوكيميائي ، تسبب المبيد في انخفاض ذى دلالة احصائية في معدل هرمون التستوستيرون ، بينما كان هناك ارتفاع ذو دلالة احصائية في مستوى كل من الهرمون المنشط لحوصلة جراف (FSH) والهرمون المنشط للجسم الأصفر (LH).

مقارنةً مع الحيوانات المعاملة بمبيد λ-سيهالوثرين ، فإن مجموعة الوقاية بمستخلص نبات السموه أظهرت وبشكل ملحوظ قدرتها على استعادة التغير في وزن الجسم والخصية ، جودة الحيوانات المنوية و معدل الهرمونات الجنسية. غير أن المجموعة العلاجية بمستخلص النبات كانت أقل فعالية في استعادة التغيرات المورفولوجية والبيوكيميائية الناجمة عن التعرض للمبيد. في حين تبين أن حقن الخلايا الجذعية كعلاج بعد التعرض للمبيد أظهر تحسنا ملحوظا في وزن الجسم والخصية ، جودة الحيوانات المنوية ، وكذلك في مستوى ونشاط الهرمونات الجنسية أكثر من استخدامها كوقاية والتي أظهرت الحد الأدنى من الحماية ضد مبيد λ-سيهالوثرين.

من النتائج السابقة يمكن استنتاج أن الوقاية بمستخلص نبات السموه والعلاج باستخدام الخلايا الجذعية كانت أكثر كفاءة من العلاج بمستخلص النبات أو الوقاية بالخلايا الجذعية في تحسين تلف الخصية الناجم عن التعرض لمبيد λ-سيهالوثرين ورفع مستوى الخصوبة والوظائف التناسلية.