Role of Mesenchymal Stem Cells in the Treatment of Testicular Toxicity Induced by Lambda-Cyhalothrin in Rats

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ABSTRACT

The present study aimed to investigate the therapeutic effects of mesenchymal stem cells (MSCs) on lambda-cyhalothrin (LCT)-induced testicular toxicity in rats. Thirty adult male rats were divided into three groups: group I: normal control; group II: received LCT (1/10DL₅₀ = 6.23 mg/kg body weight (b.w.), intraperitoneally (i.p.)); group III: received LCT (6.23 mg/kg b.w.)+MSCs (1x10⁶ cells/animal, via the tail vein). LCT elicited significant (p<0.001) declines in the serum testosterone, protein profile, testicular glutathione (GSH) and superoxide dismutase (SOD) activity and the anti-inflammatory cytokine; interleukin-10 (IL-10), whereas, serum follicle stimulating hormone (FSH), luteinizing hormone (LH), lipid profile and pro-inflammatory cytokines, tumor necrosis factor (TNF-α) and interleukin-12 (IL-12) levels were significantly (p<0.001) increased as compared to the control group. Light microscopic examination showed abnormal spermatogenic cells that displaying cytoplasmic vacuolization and nuclear pyknosis or karyolysis. Maturation arrest, hyalinization of the seminiferous tubules and congestion of the interstitial blood vessels were also observed. Ultrastructure studies confirmed the light microscope results. Compared to LCT-group, LCT+MSCs-treated rats showed significant improvement in the activity of all biochemical parameters and marked preservation in the histological and ultrastructural configuration of the testis. Conclusion: Thus therapy with MSCs was effective in ameliorating LCT-induced testicular damage by improvement of testicular function and structure as well as male fertility.

Keywords: Lambda-cyhalothrin, Mesenchymal stem cells, Rat, Testis, Biochemistry, Microscopic study
1. INTRODUCTION

Lambda-cyhalothrin (LCT), a new generation type II synthetic pyrethroid insecticide, has extensive usages 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 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., 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).

Development of stem cell technologies for cell replacement therapy has progressed rapidly in recent years (Millman and Pagliuca, 2017). MSCs are stromal cells that have the ability to self-renew and also to differentiate into mesodermal and non-mesodermal cell lineages, including osteocytes, adipocytes, chondrocytes, myocytes, cardiomyocytes, fibroblasts, myofibroblasts, epithelial cells, and neurons (Liu et al., 2009a). The beneficial effects of MSCs transplantation to the different organs are being tested clinically for curing various diseases (Monsefi et al., 2013). MSCs secrete a number of growth factors and cytokines, and also supply autocrine, paracrine, and juxtacrine factors that influence the cells of the microenvironment (Liu et al., 2009b). Recent studies reveal that the treatment of diseases, like male infertility and testosterone deficiency, was possible by adult stem cells (ASCs) (Cakici, et al., 2013).

The current study was designed to analyze the testicular toxicity caused by LCT in male rats and to elucidate the therapeutic effects of MSCs derived from the bone marrow (BM) in alleviating the detrimental effect of LCT on male fertility.

2. MATERIALS AND METHODS

2.1. Animals

Thirty 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 reviewed and approved by Research Ethical Committee of Laboratory Animals of Cairo University.
2.2. Chemicals

Lambda-cyhalothrin (LCT) with the empirical formula \((\text{C}_{23}\text{H}_{19}\text{ClF}_{3}\text{NO}_{3})\) 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 \(1/10\) \(\text{LD}_{50}\) (6.23 mg/kg/b.w.) (Fetoui et al., 2015).

2.3. Isolation and culture of bone marrow mesenchymal stem cells (BM-MSCs)

Mesenchymal stem cells (MSCs) have been isolated from bone marrow and cultured in Tissue Culture Lab, Hematology and Bone Marrow Transplantation Unit, Pediatric Hospital (EL-Mounira), Cairo University. Ten adult male Wistar albino rats with average weight 200-300g were used to prepare the MSCs by the modified method from Soleimani and Nadri (2009). Under aseptic conditions, bone marrow was harvested by flushing the tibiae and femurs of adult rats with Dulbecco’s Modified Eagle’s Medium (DMEM, GIBCO, USA). Mononuclear cells were cultured in DMEM containing 15% fetal bovine serum (FBS; Sigma), 2mm L-glutamine (Gibco, USA), 100 u/ml penicillin (Sigma) and 100 u/ml streptomycin (Sigma). Cultures were kept in a humidified incubator at an atmosphere of 5% CO2 at 37 °C for three days. Non adherent cells or hematopoietic stem cells (HSCs) were removed after three days by changing the medium and replacing with fresh complete medium. Adherent cells (passage 0) were washed with sterile phosphate buffered saline (PBS) and fresh medium was added every 3–4 days. MSCs were distinguished from other bone marrow cells by their adhesiveness to tissue culture plastic and fusiform shape (Muñoz-Fernández et al., 2006). Within 4–8 days, the culture becomes more confluent and reaches 70%-80% confluence within 2 weeks. The cultured cells were released with 0.5 ml of 0.25% trypsin/1mM ethylenediaminetetraacetic acid (EDTA) (GIBCO, USA) for 5 min at room temperature (passage 1). At this point, the next passages were initiated, and the cells were pooled and cultured (Falcon; Gibco) as passage-2 cells. The cells were subsequently expanded by several passages.

2.4. Cell viability analysis

The viability of MSCs was detected by adding equal volumes of the cell suspension and 0.4% trypan blue dye (1:1) and counting with a hemocytometer. The blue staining of cells after mixing was used as an indicator of cell death.

2.5. Flow cytometry

After the separation process, and at the 2nd passage of cell culture, flow cytometric analyses were carried out on a Fluorescence Activated Cell Sorter (FACS) flow cytometer (Coulter Epics Elite, Miami, Florida, USA). The following markers were
selected for the bone marrow stem cells identification: mesenchymal stem cell markers (CD44, CD105) and hematopoietic cell markers (CD34 and CD45).

2.6. Differentiation assays
The potential of the isolated cells to differentiate into adipogenic, osteogenic, and chondrogenic lineages was examined by incubating the cultured cells at passage 2 in appropriate induction media described by Nadri et al. (2007). The cultures were incubated at 37°C and 5% CO2 for 21 days, with medium change at 3 day intervals. Adipogenic, osteogenic and chondrogenic differentiation was assessed by Oil red O, Alizarin red and Toluidine blue staining, respectively (Soleimani and Nadri, 2009).

2.7. Experimental design
Rats were divided into 3 groups (10 animals each) as follows: Group I (control): animals received vehicles of LCT (olive oil, i.p., 8.30 ml/kg b.w., about 1ml/animal), throughout the course of 10 weeks; Group II (LCT group): rats were given LCT at a dose level of 6.23 mg/kg b.w., i.p. (6.23 mg in 8.30 ml olive oil/kg, about 0.75mg/animal according to its weight) only three times a week for two weeks and left for the next eight weeks; Group III (LCT+MSCs-treated group): rats received LCT (6.23 mg/kg b.w., i.p.) three times a week for two weeks, then injected with a single dose of MSCs (1x10⁶ cells/ml PBS/animal) via the tail vein (Zuo et al., 2013) and left for the next eight 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 chemical analysis. All animals were sacrificed and the testes were immediately excised, washed in ice-cold saline (0.9% NaCl), one testis was separated and used for microscopic examination. Serum and the other testis samples were stored at -20°C until analysis.

2.8. Detection of mesenchymal stem cells (MSCs) in the testicular tissues
Testis sections were stained with Prussian blue stain (Ellis 2007) for the demonstration of iron oxide-labeled mesenchymal therapeutic stem cells in the testicular tissue.

2.9. Physiological analysis
Determination of serum testosterone, FSH, and LH hormones was estimated depending on the assays depicted by Jaffe and Behrman (1974); Santner et al. (1981); Nankin and Troen (1972), respectively. Total protein and albumin levels in serum were determined colorimetrically according to Henry et al. (1974) and Doumas et al. (1997), respectively. Serum cholesterol and triglycerides levels were measured enzymatically by the method of Seidel et al. (1983) and Fossati and Prencipe (1982),
respectively. Serum TNF-α, IL-10, and IL-12 were determined according to the method of Aggarwal and Natarajan (1996); Okura et al. (1998) and Trinchieri (1998), respectively. GSH was assayed spectrophotometrically by the method of Tietze (1969). The activity of SOD was determined by assessing the inhibition of pyrogallol autooxidation (Nishikimi et al., 1972). MDA was determined using the method of Botsoglou et al. (1994).

2.10. Microscopic examination

For light microscopic (LM) examination, the testicular specimens were fixed in 10% neutral buffered formalin. After a proper fixation for 48 h, the tissue was cut into thinner pieces. The samples were dehydrated, cleared, and embedded in paraffin, sectioned on a rotary microtome at 4-6 μm thickness, and stained using hematoxylin and eosin (H&E) method described by Drury & Wallington (1980). Stained sections were viewed under a light microscope (Leica DM LS2, Germany).

For transmission electron microscopic (TEM) study, small pieces (about 1mm³) from the testis were rapidly cut and immediately fixed in 5% glutaraldehyde buffered at pH with 0.1M sodium cacodylate for 4 h at room temperature. Specimens were then post-fixed in 2% osmium tetroxide for 1h at 4°C, washed three times for 15 min each time with 0.1M sodium cacodylate buffer and dehydrated in ascending series of ethyl alcohol. 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 h. Semithin sections (1μm) were cut by an ultramicrotome (Leica Ultracut UCT; Germany) using a glass knife, stained with 1% toluidine blue, and examined with a light microscope to select the suitable areas for trimming the blocks. Ultrathin sections (60–80 nm thickness) were cut and double stained with uranyl acetate and lead citrate (Mascorro and Bozzola, 2007). 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.

2.11. 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. RESULTS

3.1. Morphological analysis of MSCs in primary culture
The cell culture during the first two days was heterogeneous containing round and non-adherent cells. At the 5th day, the cell population became more homogenous presenting an adherent fibroblastoid appearance and formed cell colonies. The cells reached confluence and appeared as long spindle-shaped cells on days 9–14, and were attached to the culture flasks (Figure 1).

3.2. Assessment of cell viability
The viability of MSCs was confirmed by Trypan blue dye. The viable cells were not stained, and the dead cells were stained blue (Figure 2).

3.3. Immunophenotypic characterization of MSCs
The following markers were selected for bone marrow stem-cells identification: MSCs markers (CD44, CD105) and hematopoietic cells markers (CD34 and CD45). According to size (FSC) and complexity (SSC) parameters, a population of cells was observed, and flow cytometry analysis showed that the 2nd passage cells were positive for CD44-PE (69.7±4%) and CD105-PE (73.5±6%), but negative for CD45-CY5 (6.6±2%), while moderate expression has been reported for CD34-FITC (17±1%) (Figure 3).

3.4. Differentiation assay of MSCs
After incubation with the adipogenic supplementation, small lipid droplets appeared within the cytoplasm of the cells first on days 6–7; they gradually occupied the whole cells, by day 21, indicating the adipogenic capacity of these cells, which was confirmed by Oil red O staining (Figure 4A). The MSCs cultivated in the osteogenic medium showed morphology similar to osteoblasts after 21 days of stimulation, with the presence of calcium precipitates, confirmed by Alizarin red staining (Figure 4B). When the MSCs were cultivated in the chondrogenic medium during 21 days, the cells showed similar morphology to chondrocytes, evidenced by Toluidine blue staining (Figure 4C).

3.5. Detection of MSCs in testicular tissue after the transplantation
Sections in the testes of control rats showed Prussian blue negative-stained cells (Figure 5A). LCT+MSCs-treated group showed spindle-shaped, branched, and globular-shaped Prussian blue positive-stained cells between spermatogenic cell layers (Figure 5B) and inside the lumen of the seminiferous tubules (Figure 5C).
3.6. Physiological analysis

Treatment with LCT resulted in a significant (p<0.001) decrease in serum levels of testosterone, total protein, and albumin content, while caused a significant (p<0.001) increase in the levels of FSH, LH hormones, cholesterol and TG as compared with normal control rats. In contrast, administration of MSC in LCT+MSCs-treated group significantly alleviated the distortion in the aforementioned biochemical parameters, as compared with that of LCT group (Table 1).

LCT group exhibited a significant (p<0.001) elevation in the pro-inflammatory cytokines, TNF-α, IL-12 and testicular MDA, along with a significant (p<0.001) decrease in the anti-inflammatory cytokine; IL-10 level, testicular GSH and SOD as compared to control group. The administration of MSC in LCT+MSCs-treated group significantly preserved the level of these parameters to near normal levels when compared with LCT-intoxicated rats (Table 2).

3.7. Microscopic studies

3.7.1. Light microscopic study

Evaluation of hematoxylin and eosin-stained testicular sections of control rats revealed the normal histoarchitecture of well-organized seminiferous tubules including all stages of the spermatogenic cycle and normal interstitial connective tissue (Figure 6A).

Inspection of testis sections of rats of the LCT-treated group revealed severe damage of the seminiferous tubules with nuclear pyknosis or karyolysis of the spermatogenic cells and separation of these cells from the underlying layer of the tubules (Figure 6B). Vacuolation of the cytoplasm of most of the spermatogenic cells, maturation arrest, hyalinization of the seminiferous tubule, and exfoliation of necrotic cells in the tubular lumina were also observed (Figure 6C). In addition, congested dilated blood vessels were seen in the interstitium (Figure 6B).

Light microscopic preparation of testis sections of rats of LCT+MSCs-treated group revealed improvement in the seminiferous tubule structure with nearly restoration of spermatogenesis to its normal situations compared to the LCT group (Figure 6D). Spermatogenic layers were comparatively well-organized and spermatozoa were observed in the tubular lumina (Figure 6D). However, few spermatogenic cells with pyknotic or karyolytic nuclei were still observed. (Figure 6D).

3.7.2. Transmission electron microscopic study

Electron microscopic examination of ultrathin sections of the testis of control rats revealed that each seminiferous tubule is coated with the boundary tissue which consists of a thin layer of collagen fibrils between the basal lamina and the myoid cells. The inner and outer surfaces of the myoid cells are also surrounded by
discontinuous and continuous basal laminae, respectively (Figure 7A). Sertoli cells have large pale nuclei and prominent nucleoli. Spermatogonia appear with rounded nuclei resting on the basal laminae. Spermatocytes have large euchromatic nuclei with a thin rim of cytoplasm containing mitochondria (Figure 7A). Early differentiating spermatids contain numerous peripherally arranged mitochondria and developing acrosomes that appeared as acrosomal granules and acrosomal vesicles. Acrosomal caps, resulting from spreading of the acrosomal vesicles, appeared covering one hemisphere of the nuclei of differentiating spermatids (Figure 7B). Near the lumen, late elongated spermatids with strongly elongated condensed nuclei covered anteriorly by the acrosomal caps are detected (Figure 7B).

Electron microscopic examination of ultrathin sections of the testis of LCT-treated rats showed marked irregularity and enfolding of the basal laminae and thickened, deformed boundary tissue (Figure 8A). Spermatogonia and Sertoli cells showed abnormal nuclei with a highly electron-dense chromatin. The cytoplasm was degenerated and contained few cell organoids and vacuoles of various sizes (Figure 8A). The same group exhibited abnormal primary spermatocytes with necrotic changes. Some spermatocytes displayed karyolysis and cytoplasmic disintegration, while the others showed irregular shrunken nuclei and cytoplasmic vacuolization (Figure 8B). Marked edema and necrotic debris in between the highly destroyed spermatogenic cells can also be seen (Figure 8B). Spermatids displayed damaged nuclei with irregular outlines, distorted acrosomes, vacuolated degenerated mitochondria and an increase in the number of lysosomes (Figure 8C).

The ultrastructure of ultrathin sections of the seminiferous tubules of the LCT+MSCs-treated rats showed marked restoration of the general structure of testicular tissues. Sertoli cells had nearly intact nuclei; however, their cytoplasm showed an increase in the number of lysosomes (Figure 9A). The boundary tissue was slightly disrupted in certain areas and intercellular separations still be recognized between some cells (Figure 9A). Primary spermatocytes appeared with large nuclei and normal cytoplasmic organelles, only a few primary spermatocytes lied in close contact with the basal laminae of the tubules (Fig. 9B). Round spermatids had well-formed acrosomes (Figure 9B).

4. 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.

Physiologically, 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. 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 and El-Maddawy (2012) who observed a significant reduction of serum testosterone level in deltamethrin (a type II synthetic pyrethroid insecticide)-treated rats. Moreover, decreased testosterone synthesis following LCT administration might be associated with down-regulation of steroidogenic acute regulatory protein (StAR) in the testis. StAR is essential for 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 cytochrome P450 side-chain cleavage enzyme (P450scc), 17α-hydroxylase-C17,20-lyase (P45017α), and 17β-hydroxysteroid dehydrogenase (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 P45017α to produce 17-hydroxypregesterone and androstenedione, with the latter then being converted to testosterone by 17β-HSD (Payne and Youngblood, 1995).

Also, 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. LCT+MSCs-treated group showed a significant improvement in the changes of T, FSH and LH reached to near normal levels as compared with that of LCT rats. This improvement may be due to the fact that MSCs could differentiate into germ cells. These findings are in agreement with the results observed by Abd El-Dayem et al. (2015) who reported that transplantation of MSCs markedly restored serum testosterone, FSH and LH hormones. This may be due to the capacity of MSCs to differentiate into steroidogenic cells, such as Leydig cells, both in vivo and in vitro (Yazawa et al., 2006). Also, Cakici et al. (2013) revealed that MSCs were found both outside of the basal compartment and in the seminiferous tubules, supporting the idea that MSCs have functioned in the restoration of spermatogenesis by differentiation into sperm or maintenance of the spermatogonial stem cells. Therefore, MSCs could be both a rich and functional source for infertility treatment.

LCT induced depletion in serum total protein and albumin levels compared to control rats. This may be attributed to the destruction/necrosis of cells and a reduction of the protein synthesis in the liver and clearly reflect the abnormalities in lipoprotein metabolism. These results are harmony with the results of Morgan and Osman (2007) and Parthasarathy and Joseph (2011). The current work found that animals treated by MSCs recorded a significant increase in the content of both albumin and total protein compared with LCT group. These effects may be, at least partly, due to the capability of stem cells to give repopulation to hepatocytes. The hepatoprotective effects of stem cells are confirmed by studies reported by Lagasse et al. (2000) who found that stem cells may be a source of hepatocytes for therapeutic liver repopulation in the
appropriate conditions. Furthermore, stem cell-based therapy has been recognized as a promising treating strategy of a variety of diseases, including liver disorders. For example, BM-MSCs provide protection against liver injury by the antioxidative process, vasculature protection, hepatocyte differentiation, and trophic effects (Francois et al., 2013).

Moreover, LCT treatment caused a significant elevation in serum levels of cholesterol and TG. These results are in agreement with Abu-Aita and Yassa (2008) who noticed hyperlipidemia with significantly elevated levels of serum TG and cholesterol in deltamethrin exposed rats. This could be a result of the diseased liver by administration of the insecticide (Owen, 1990). In the present experiment, improvement occurred in lipid profile levels in LCT+MSCs-therapeutic group. This finding may be due to modulatory influence on lipogenic enzymes or by inhibition of cholesterol absorption (El-Khawaga et al., 2010). Hypocholesterolemic effect of MSCs can be as a result of a direct effect on liver and kidney or an indirect effect through thyroid hormones which affect in the lipid metabolism and it is known that MSCs are a promising therapeutic tool for diseases, such as liver failure and kidney injury (Gad et al., 2017).

Administration of LCT 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 a negative association between levels of permethrin insecticide and levels of anti-inflammatory cytokine component. The results obtained in the present study demonstrated that combined administration of MSCs plus LCT to rats offered a significant improvement by enhancing IL-10 expression and inhibiting TNF-α and IL-12 expression, which provided a possible link between these three cytokines and MSCs. Results of the present study are in accordance with the findings of Liu et al. (2009b) who reported that the reduction of cerebral ischemic injury by MSCs may be partly caused by inhibition of TNF-α and IL-12 and elevation of IL-10. The authors also illustrated that a possible tie between the MSCs and parenchymal cell local production of growth factors may be related to the ability of MSCs to produce a wide variety of trophic factors and cytokines, some of which activate the production of IL-10 and decrease the production of TNF-α. For instance, MSCs can secrete basic fibroblast growth factor (bFGF), and bFGF decreases expression of TNF-α (Zhang et al., 2005).

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 group as compared with the control group. 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 such as superoxide radical (O₂⁻), and hydroxyl radical (OH⁻) thus causing failure of antioxidant defense system to overcome the influx of ROS induced by LCT exposure (Fetoui et al., 2010; Madkour, 2012). However, administration of
MSCs extract significantly prevented the influence of LCT on the antioxidative system. It decreased MDA and concomitantly increased the activity of SOD and GSH content in testicular tissue. These findings go side by side with the results obtained by Burova et al. (2013) who reported that the in vivo protection by BM-MSCs against lead-induced oxidative damage may be because of its free radical scavenging potential. The specific responses of MSCs to oxidative stress may play a crucial role in the regulation of tissue homeostasis as well as regeneration of organs after oxidative injury. It could also be because of direct scavenging/neutralization of the free radical or induction of endogenous antioxidant enzymes, such as CAT and SOD (Hassan and Alam, 2014). Moreover, transplantation of MSCs can correct and reverse the imbalance between ROS and antioxidant defense in favor of antioxidant defense by restoring and augmenting its capacity as well as modulating lipid peroxidation (El Far et al., 2012).

Regarding the light microscopic examination, LCT provoked some histopathological changes in the testis such as degeneration, exfoliation of necrotic cells, maturation arrest and hyalinization of the luminal content. Similar results have been observed in rats (Oda and Maddawy, 2012; Ben Abdallah et al., 2013), mice (Abdel Aziz and Abdel Rahem, 2010; Al-Shaikh, 2013; Al-Sarar et al., 2014) and fish (Parthasarathy and Joseph, 2011; Xia et al., 2016) after pyrethroid insecticides administration. In view of the fact that Sertoli cells are the supportive cells within the seminiferous tubules and provide multitude factors required for spermatogenesis (Russell, 1993). Consequently, the detachment of spermatogenic cells from the underlying layer pointed out the Sertoli cell damage due to microtubule impairment (Kumar et al., 2006). Also, abnormal exfoliation of the degenerated spermatogenic cells could be due to a primary effect on the cell-to-cell junctions between Sertoli and germ cells. 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). By electron microscopy, as a consequence of LCT administration, the seminiferous tubules showed marked irregularity of basement membrane and thickened, distorted boundary tissue. Abnormal spermatogenic cells with vacuolated cytoplasm, deformed spermatids, in addition to a highly degenerated Sertoli cell with an overall decrease in cytoplasmic organelles were also observed. Similar findings were 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 spermatogenic 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.
The present investigation suggested that structural alterations of the testis may be attributed to LCT-induced lipid peroxidation (LPO) which was recorded in serum and testes tissue. In this respect, Aitken et al. (1993) reported that 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. The increased LPO can lead to oxidative damage to sperm DNA, alter membrane functions, impair motility and possibly have a significant effect on the development of spermatozoa (Aitken et al., 1989).

Using Prussian blue stained-sections in animals of the LCT+MSCs-treated group showed spindle-shaped, branched, and globular-shaped MSCs between the layers of the seminiferous tubules, indicating migration of injected stem cells to the injured testis. This stem cell-treated group revealed slightly affected testicular tissue and cell ultrastructure with nearly normal spermatogenesis in some tubules after 8 weeks. The results clearly showed that MSCs can reconstitute the tubular microenvironment by producing germinal cells within the host seminiferous tubules. This can be seen because the injected MSCs differentiated to testicular germinal cells in their new niche and this confirms that stem-cell therapy could help the fast repair of pathological changes in testicular seminiferous tubules. These findings are reinforced by many authors. Nayernia et al. (2006) published positive results using BM-MSCs transplanted into degenerated testicles. Also, Lue et al. (2007) showed that BM-MSCs, transplanted into testis of a busulfan-induced infertile male mouse model, appeared to differentiate into germ cells, Sertoli cells, and Leydig cells. More recently, Cakici et al. (2013) demonstrated that, in busulfan-induced infertile male rats, the testis appeared morphologically normal with spermatogenesis in some tubules of MSCs-injected testes. In addition, Monsefi et al. (2013) reported that the MSCs transplanted to their new niche of atrophic testicular seminiferous tubules of busulfan-treated male rats could survive and settled down in the seminiferous tubules and interstitium. Spermatogonial stem cells (SSCs) that originated of MSCs proliferated and produced other germ cells of primary spermatocyte, spermatids, and spermatozoa in some seminiferous tubules of recipient’s rats. Also, MSCs existed in the interstitial connective tissue between the seminiferous tubules and their morphology was like Leydig cells. The authors illustrated that these findings may be also due to either proliferation of live SSCs by growth factor that was produced by MSCs or direct differentiation of MSCs to spermatogonia. The blood-testis barrier may help the MSCs to preserve from immunologic responses (Lue et al 2007). The authors added that MSCs did not reveal in all testicular seminiferous tubules may be due to the normal repair of germinal epitheliums after busulfan injection. There are many investigators who discussed the mechanisms of MSCs in tissue repairing. Stem cells may also act by suppressing inflammation in the diseased organ via up-regulating anti-inflammatory cytokine (IL-10 expression) (Liu et al., 2009b) and down-regulating of pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) (Wang et
al., 2006). Both of these processes are thought to contribute to the regeneration of cells in the damaged organ (Pai et al., 2012).

Another possible explanation for regeneration and improvement in target organ structure and function appear to be through an increase in the production of the cytokine vascular endothelial growth factor (VEGF) by MSCs. Increased VEGF could involve in or trigger angiogenic progress of MSCs, even might contribute to neovascularization by mobilizing bone marrow-derived endothelial progenitor cells (Asahara et al., 1999). This finding leads to improvement of blood supply to the cells and thereby helps to repair damaged tissue (Tang et al., 2006).

In the present work, significant improvement in spermatogenesis was observed after injection of MSCs into the tail vein of LCT-treated rats. However, more significant improvement with complete spermatogenesis was confirmed by Qu et al. (2012) following transplantation of SSCs into testes of mice after depletion of their own germ cells by means of busulfan; this may be due to the fact that the transplantation of SSCs stimulated endogenous spermatogenesis in the recipient mice.

5. CONCLUSION

The present study revealed that MSCs transplantation can improve testicular structure and function in lambda-cyhalothrin-intoxicated rats. More studies are needed to shine a light on the potential clinical benefit of adult stem cell therapy for improvement of male fertility and reproductive functions and also to demonstrate the mechanisms of engraftment, homing, in vivo differentiation and the long-term safety of these therapies.

6. REFERENCES


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damage with concomitant decrease in testosterone and increase in lactate dehydrogenase concentration in the testis. Mutation Research, 607(2), 240-252.


Table 1: The therapeutic role of MSCs on serum sex hormone levels, protein profile and lipid profile in LCT-intoxicated rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Control</th>
<th>LCT</th>
<th>LCT+MSCs</th>
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<tr>
<td>Testosterone ng/ml</td>
<td></td>
<td>13.31±0.96</td>
<td>2.80±0.78 **</td>
<td>9.71±0.59 a b **</td>
</tr>
<tr>
<td>FSH ng/ml</td>
<td></td>
<td>2.65±0.13</td>
<td>3.78±0.14 a **</td>
<td>3.05±0.05 **</td>
</tr>
<tr>
<td>LH ng/ml</td>
<td></td>
<td>1.92±0.01</td>
<td>2.93±0.09 a **</td>
<td>2.23±0.01 a b **</td>
</tr>
<tr>
<td>Total protein g/dl</td>
<td></td>
<td>8.74±0.30</td>
<td>4.96±0.12 a **</td>
<td>7.03±0.27 a **</td>
</tr>
<tr>
<td>Albumin g/dl</td>
<td></td>
<td>5.13±0.37</td>
<td>1.94±0.06 a **</td>
<td>3.64±0.20 a **</td>
</tr>
<tr>
<td>Cholesterol mg/dl</td>
<td></td>
<td>62.22±2.54</td>
<td>105.40±4.67 a **</td>
<td>70.70±2.58 b **</td>
</tr>
<tr>
<td>TG mg/dl</td>
<td></td>
<td>130.04±2.43</td>
<td>213.01±3.56 a **</td>
<td>141.73±3.54 b **</td>
</tr>
</tbody>
</table>

Data are expressed as means ± S.E. (n = 10 in each group).

a: Significant change at p< 0.05 with respect to control group; b: Significant change at p< 0.05 with respect to LCT-group. * Highly significant change at p<0.01; ** very highly significant change at p<0.001

Table 2: The therapeutic role of MSCs on cytokines, antioxidants and oxidative stress biomarkers in LCT-intoxicated rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Control</th>
<th>LCT</th>
<th>LCT+MSCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α pg/ml</td>
<td></td>
<td>44.10±3.69</td>
<td>144.22±6.63 a **</td>
<td>66.95±3.92 a b **</td>
</tr>
<tr>
<td>IL-10 pg/ml</td>
<td></td>
<td>117.92±4.63</td>
<td>22.10± 2.59 a **</td>
<td>81.84± 4.5 a b **</td>
</tr>
<tr>
<td>IL-12 pg/ml</td>
<td></td>
<td>83.78±4.42</td>
<td>184.00±6.50 a **</td>
<td>112.93±6.52 a b **</td>
</tr>
<tr>
<td>GSH μg/g</td>
<td></td>
<td>12.97±0.41</td>
<td>6.90±0.29 a **</td>
<td>9.96 ± 0.28 a b **</td>
</tr>
<tr>
<td>SOD μg/g</td>
<td></td>
<td>19.36±0.54</td>
<td>9.09±0.60 a **</td>
<td>15.15±1.60 b **</td>
</tr>
<tr>
<td>MDA μmol/100g proteins</td>
<td></td>
<td>0.64±0.03</td>
<td>1.25±0.02 a **</td>
<td>0.83±0.03 a b **</td>
</tr>
</tbody>
</table>

Data are expressed as means ± S.E. (n = 10 in each group).

a: Significant change at p< 0.05 with respect to control group; b: Significant change at p< 0.05 with respect to LCT group. * Highly significant change at p<0.01; ** very highly significant change at p<0.001
Figure 1: Light micrographs showing morphological analysis of MSCs in primary culture. (A): During the onset of culture, 1-2 days, cells are round with large nuclei surrounded by cytoplasm. (B): On day 5, MSCs exhibited large, flattened or fibroblast-like morphology. (C): On days 9–14, MSCs appeared as long spindle-shaped cells. Contrast-phase inverted microscope. (A, B 100 x; C 400x)
Figure 2: Light micrograph showing post-trypsinization MSCs. Cells without the dye are the viable cells (arrows). (Trypan blue, 100x)
Figure 3: Phenotypical evaluation of BM-MSCs. Cells were stained with the CD34, CD44, CD45, and CD105 antibodies and analyzed by flow cytometry. (A): A plot of granularity (SSC) versus size (FSC), demonstrating the cell population selected for the study. (B): Graphic representation showing the percentage of fluorescent cells inside the selected population. The expression levels are positive for CD44-PE (69.7±4%) and CD105-PE (73.5±6%), but negative for CD45-CY5 (6.6±2%), while moderate expression has been reported for CD34-FITC (17±1%).[Side Scatter (SSC) and cell size in x-axis (Forward Scatter-FSC)].
Figure 4: Light micrographs showing *in vitro* differentiation of MSCs into (A): Adipocytes stained with Oil red O. (B): Osteogenic lineage (mineralizing cells) stained with Alizarin red. (C): Chondrocytes stained with Toluidine blue. (A, B 100x; C 400x)
Figure 5: Light micrographs of Prussian blue stained-sections of the testes. (A): Control rat showing Prussian blue negative-stained cells. (B): LCT+MSCs-treated rats showing homing of Prussian blue positive-stained MSCs between spermatogenic layers (arrows). (C): LCT+MSCs-treated rats showing homing of Prussian blue positive-stained MSCs inside the lumen of the seminiferous tubule (arrows). (Prussian blue, A-C 400x; Insets: 1000x)
Figure 6: Light micrograph of testis sections of control rats. (A): Showing parts of six adjacent seminiferous tubules (ST) surrounded by the boundary tissue (arrows) and separated by narrow interstitium (I) containing interstitial Leydig cells. The tubules show successive stages of spermatogenesis including spermatogonia (Sg), primary spermatocytes (PS), spermatids (Sd), and spermatozoa (SZ). The supporting Sertoli cells (SC) are observed between these cells. Light micrographs of testis sections of LCT-treated rats (B&C). (B): Showing shrunken pyknotic (arrows) or karyolytic (arrowheads) nuclei of many spermatogenic cells and detachment of these cells (double arrows) from the underlying layer. Note that the interstitium has a congested dilated blood vessel (BV). (C) Showing vacuolization (V) of the cytoplasm of most of the spermatogenic cells and maturation arrest. Note hyalinization of the seminiferous tubule (*) and plugging of its lumen with exfoliated, necrotic cells (arrow). Light micrograph of testis sections of LCT+MSCs-treated rats. (D): Showing improvement in the structure of the seminiferous tubules. However, few spermatogenic cells with nuclear pyknosis (arrow) or karyolysis (arrowheads) are still noticed. (H&E, A-D 400x)
**Figure 7:** Transmission electron micrographs of parts of the seminiferous tubules of the testes of control rats. (A): Showing a part of a seminiferous tubule surrounded by a regular basal lamina (BL) ensheathed by a flat myoid cell (MC). Spermatogonia (Sg) appear with oval nuclei and their cytoplasm contains mitochondria (M). Primary spermatocytes (PS) have large euchromatic nuclei with synaptonemal complex (arrow) and a thin rim of cytoplasm containing many small mitochondria (M). Part of the Sertoli cell (SC) with its characteristic large indented nucleus (N) and prominent nucleolus (Nu) can also be seen. Its cytoplasm shows mitochondria (M), electron-dense lysosomes (Ly), rough endoplasmic reticulum (rER) and small vesicles of smooth endoplasmic reticulum (sER). BT, Boundary tissue (TEM, 6000x). (B): Showing a group of early rounded differentiating spermatids (Sd) with the characteristic peripheral arrangement of mitochondria and acrosomal caps (AC) spreading over the rounded nuclei. Note late elongated spermatid (Sd1) with a strongly elongated condensed nucleus (N) covered anteriorly by the acrosomal cap (arrow). Gg, Golgi apparatus. (TEM, 8000x)
Figure 8: Transmission electron micrographs of parts of the seminiferous tubules of the testes of LCT-treated rats. (A): Showing marked irregularity and enfolding of the basal lamina (BL) and thickened, deformed boundary tissue (BT). Sertoli cell (SC) and spermatogonium (Sg) show abnormal nuclei (N) with highly electron-dense chromatin and few cell organoids. Note intracellular vacuoles (V) and highly widening of intercellular spaces (*) (TEM, 8000x). (B): Showing necrotic changes of the primary spermatocytes. Note karyolysis and cytoplasmic disintegration with disruption of the cell membrane of primary spermatocyte (PS1) and irregular shrunken nucleus and cytoplasmic vacuolization (V) of the other one (PS2). Marked edema (*) and necrotic debris (arrows) in between the highly destroyed spermatogenic cells can also be seen (TEM, 10000x). (C): Showing spermatids (Sd) with distorted acrosomes, irregular damaged nuclei (N), vacuolated degenerated mitochondria (M) and an increase in the number of lysosomes (Ly). (TEM, 5000x)
Figure 9: Transmission electron micrographs of parts of the seminiferous tubules of the testes of LCT+MSCs-treated rats. (A): Showing almost normal fine structure of spermatogonia (Sg) and primary spermatocytes (PS). Sertoli cell (SC) has nearly intact nucleus but its cytoplasm showed an increase in the number of lysosomes (Ly). Also, the boundary tissue (BT) is slightly disrupted (arrow) and the intercellular separations (*) are still seen between some cells (TEM, 6000x). (B): Showing more or less normal appearance of boundary tissue (BT), primary spermatocytes (PS) with a large nucleus (N) and normal cytoplasmic organelles, and round spermatids (Sd) with well-formed acrosomal caps (AC). However, the intercellular spaces are edematous (*) and the primary spermatocyte lies in close contact with the basal lamina (BL) of the tubule. (TEM, 10000x)