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It is with great pleasure that I write this editorial to welcome you to the IJCBR. This journal provides a platform for publication of original and reviews research articles, short communications, letter to editor, thesis abstract, conference report, and case studies. These types of publication are directed at the interface of the fields of cancer and biomedical research.

The IJCBR relies on a distinguished expert of the Advisory and Editorial Board Members from the top international league covering in depth the related topics. They timely review all manuscripts and maintain highest standards of quality and scientific methodology and ethical concepts. Meanwhile, we take all possible means to keep the time of the publication process as short as possible.

I take this chance to welcome your contributions to the IJCBR and have every expectation that it will soon become one of the most respected journals in both the fields of cancer and biomedical research.

Mohamed L. Salem,

Editor in Chief

INTERNATIONAL JOURNAL OF CANCER AND BIOMEDICAL RESEARCH (IJCBR)

RESEARCH ARTICLE

Adipose-derived mesenchymal stem cells ameliorate the damages induced by arsenic trioxide in the testis of rats

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ABSTRACT

Background: Arsenic trioxide is an element present everywhere in the environment. **Aim:** The current investigation planned to explain the remedial impacts of adipose mesenchymal stem cells (AD-MSCs) of rats against harmful impacts of Arsenic trioxide (As_2O_3; As III) on the testis of rats. **Materials and Methods:** Sixty male rats were divided into six groups: Group (1): served as the control without any treatments. Group (2): received a daily oral dose of HCL (0.5 mg/kg b.w). Experimental groups (3 and 4) received a low dose (3mg/kg b.w) of As III and groups (5,6) received a high dose (15 mg/kg b.w) of As III. The rats were sacrificed after 15 days in groups (3,5). Groups (4,6) were injected with (0.6×10^6) AD-MSCs / 0.5 ml PBS immediately after 15 days of oral doses of As III in low and high dose then sacrificed 7 days after MSCs injection. **Results:** The results indicated that AD-MSCs decreased the levels of p53 and Bax and increased the level of Bcl2. Additionally, treatment with AD-MSCs rescued the toxicity of low and high doses (L &H) As III induced change in TNF-α, IL-6, and IL-10 levels. In addition, As III L &H + AD-MSCs administration resulted in the gradual elevation of CD44 mRNA compared to control untreated mice. low and high doses of As III prompted histopathological damages in testis were they mostly turned around by treatment of AD-MSCs. **Conclusion:** The present results demonstrate that AD-MSCs got from the adipose tissue of rats can be a powerful treatment against harmfulness induced by As III in testis.

Keywords: Arsenic trioxide, Apoptosis, Adipose mesenchymal stem cells, Cytokines, testis injury.

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INTRODUCTION

Heavy metals like Arsenic starts cytotoxicity by presenting oxidative stress (Liu et al., 2003). Oxidative stress emerges when responsive oxygen species by Arsenic occurs in both natural and inorganic structures. In nature yet inorganic types of arsenic [As III and As V] are considered to be a likely danger to the environment, humans and other organisms because of their cancer-causing and different bad impacts (Singh and Pant, 2004). The attributes of intense arsenic poisonousness incorporates harmful effects on the gastrointestinal (Hughes, 2002), neoplastic changes of the skin and respiratory tract, haematopoietic, cardiovascular, anxious, hepatic, endocrine and kidney (Wang et al., 2006). Different experiments have indicated severe gonadal harm in different animals during spermatogenesis following exposure to heavy metals (Mandal and Das, 2012; Mahajan et al., 2018). In addition, arsenic is a notable human cancer-causing agent (Balakumar et al., 2010).

It has been reported that inducing oxidative stress by arsenic has a toxic effect on the testis of the mice finally leads to a gradual decrease in the spermatic tube diameter (Sanghamitra et al., 2008 and Mehrzadi et al., 2018). Also arsenic induces distortions in the male reproductive system and spermatotoxicity in many animals (Sarkar et al., 2003; Pant et al., 2004 and Chang et al., 2007). Likewise, arsenic may actuate hypogonadism through the decline of testosterone and apoptosis (Shen et al., 2013 and Zubair et al., 2017). Arsenic trioxide has proficiently prompted apoptosis in the cancer cells in vitro by the exposure of some malignant cells to As III produces reactive oxygen species and decrease of p53 level using both caspase-

depended and non-depended pathways (Yoon-Jae Kim et al., 2011).

Bone marrow has adult stem cells known as mesenchymal stem cells (MSCs), which are characterized as pluripotent cells. These cells can differentiate into multiple mesodermal cells. MSCs must express CD73, CD105 and CD90, and no expression of CD45, CD34, CD14 or CD19 (Dominici et al., 2006 and Malihezaman Monsefi et al., 2013). MSCs are stromal cells that can self-reestablish and separate into mesodermal and nonmesodermal cell ancestries, including osteocytes, adipocytes, chondrocytes, myocytes, cardiomyocytes, fibroblasts, myofibroblasts, epithelial cells, and neurons (Millman and Pagliuca, 2017). MSCs has unique features, their transplantation can improve different diseases. In rat stroke models, after 7 days of bloodstream restriction to the brain, MSCs injection leads to restoration of blood flow (Jung et al., 2011). MSCs secreted enormous amounts of bioactive components that are both immunomodulatory and trophic. The trophic action activated mitosis of tissue progenitor cells (Melanie et al., 2012). Likewise, MSCs can improve the germinal epithelial potential in testicular seminiferous tubules in male rats (Malihezaman Monsefi et al., 2013).

Effective utilization of MSCs in a few animals models improves diverse organ harmfulness (Lim et al., 2016 and Wang et al., 2017) evoked the direction of the current examination to assess AD-MSCs against testicular harm prompted by As III L&H doses. MSCs separated from adipose tissue has more proliferative limit than BM-MSCs (Zuk, 2010). As of late, the researchers began to look for another source for MSCs instead of the bone marrow because of MSCs from the latter yield low cell number upon harvest (Zuk et al., 2002). Adipose tissue-derived mesenchymal stem cells (AD-MSCs) are gotten from the mesenchyme which contains strong stroma that is easily disengaged. In this manner, it is believed that adipose tissue may represent a rich source of stem cells that could have effects on many fields (Atalla et al., 2018).

Emerging proof has built up that MSCs and AD-MSCs can repair and recover tissues and the valuable impacts of MSCs transplantation to the various organs are being tried clinically for relieving different sicknesses (Monsefi et al., 2013). MSC can rectify tissues, through cytokines, apoptosis inducers, autocrine, paracrine, juxtacrine factor. They can also be hereditarily built to create antitumor particles for example interferon β (INF β) and TNF-related apoptosis-inducing ligand (TRAIL) (Li Z et al, 2015; Metwall et al., 2017 and Serhal et al., 2019). Along these lines, the current investigation intended to show the conceivable restorative impact of AD-MSCs against arsenic-induced testicular toxicity in male rats.

MATERIALS AND METHODS

Drug and animals

As III from (Sigma-Aldrich, St. USA). A stock solution of Arsenic trioxide was prepared by dissolving it into 0.12 N HCL (0.5 mg/kg b.w). Two doses of Arsenic trioxide (3 &15 mg/kg) were used in the current work. Sixty adult male Wistar rats weighting 210±50g were purchased from Assiut University Joint Animal Breeding Unit. Rats were kept in 23±2ºC and a lighting cycle of 12 hours light /dark. The procedures of the experiment were conducted in strict compliance with ethics prepared by INSA and (WHO/UNESCO).

Experimental design

Sixty male rats were divided into six groups, each group consists of 10 rats: Group (1): served as a control without any treatments. Group (2): received a daily oral dose of 0.12 N HCL (0.5 mg/kg b.w). Experimental groups (3,4) received a low dose (3 mg/kg b.w) of As III. Experimental groups (5,6) received a high dose (15 mg/kg b.w) of As III. The rats were sacrificed after 15 days in groups (3,5). In groups (4,6) each rat was injected with (0.6×10^6) AD-MSCs / 0.5 ml PBS via caudal vein immediately after 15 days of oral doses of As III then they were sacrificed 7 days after AD-MSCs transplantation.

Methods

Isolation of AD-MSCs from rats

AD-MSCs were isolated from the visceral fats of adult rats. Fats were taken and cut into small pieces at a range of 1–3 mm. To remove the remaining blood from the adipose tissues, the latter was washed three times with sterile phosphate buffer solution. Adipose tissue pieces were enzymatically digested with 0.25%

Collagenase type II in PBS with 20% FBS for 45-60 minutes at 37°C with shaking every 15 min. The collagenase activity was stopped by 5 ml of fetal bovine serum. After that centrifugation for 10 min (1800 rpm, 25 °C), the supernatant was discarded and pellets were suspended in 12 ml of DMEM culture medium. The suspension Filtrated through a cell strainer (40 µm) into a fresh 50 ml falcon tube. Then transferred the cell suspension into a culture flask and incubated at 37°C with 5% Co2. The medium was changed three times a week, and The cells were grown Approximately for 15 days until almost confluency 80%. 3rd passages of MSCs were suitable for transplantation (Harsan et al., 2015).

Morphological Characterization of AD- MSCs

AD-MSCs were observed under an inverted microscope, at passage 1 they have had a fibroblast-like morphology.

Analysis of Cell Surface Markers of AD-MSCs by flow cytometry

After the third passage, the undifferentiated AD-MSCs were subjected to flow cytometer analysis to detect the following antigens: CD105, 44 and 14 by using monoclonal antibodies specific for each antigen. Adherent cells were adjusted to trypsinization with 0.25% trypsin/ EDTA. 1×10^5 cells/ml were then fixed for 30 min in ice-cold 2% formaldehyde. The fixed cells were washed in flow cytometry buffer then incubated for 30 min in the buffer of flow cytometry containing fluorescein isothiocyanate-conjugated monoclonal antibodies: CD105, 44 and CD14 IgG (Beckman coulter, USA) at 4º C in the dark. Then 2 ml of PBS solution containing 2% FBS were added to each tube of monoclonal treated cells. Cells were centrifuged and re-suspended in 0.5 ml of PBS containing 2% FBS solution. CYTOMICS FC 500 Flow Cytometer (Beckman Coulter, FL, USA) was used for Cell analysis with CXP Software version 2.2. Flow cytometry analysis of AD-MSCs revealed positive expression of CD105, 44 and negative expression of CD14.

Western blot analysis

For AD-MSCs characterization, the third passaged AD-MSCs were harvested using trypsin/EDTA digestion and suspended at a concentration of 0.6×10^6 cells/100 µl in PBS. Cells were lysed and equal protein amounts were subjected to SDS PAGE electrophoresis and then the protein band was transferred onto a nitrocellulose membrane. Monoclonal IgG antibodies against cd 105, 44 and 14 were applied and then secondary anti-mouse IgG HRP conjugated antibody was applied. Immunoreactive bands were visualized by enhanced chemoluminescence substance. Actin was used as an internal control for protein equal loading. CD 105 and CD 44 are positive markers for stem cell and CD 14 is a positive marker for leukocytes but a negative marker for stem cell. Western blot analysis for the evaluation of P53, Bax and BCL2 levels in testis after different treatments were carried out in a similarly as described above except for some modifications. samples of testis were homogenized in RIPA Lysis buffer (Nonidet-P40 1%, TritonX-100 1%, Na dioxycholate 0.5%, NaCl 150 mM, EDTA 5 mM, EGTA 10 mM, Tris-HCl 50 mM., PMSF 1 mM, protease inhibitor). Samples were resolved by 10% SDS-PAGE and transferred onto nitrocellulose membrane. Block the membrane with 5 % skim milk in TBS with 0.05% Tween 20. Incubation overnight at 4°C with primary antibodies (mouse anti-p53, anti-BAX & anti-BCL2 IgGs) then incubated for 1h at ambient temperature with anti-mouse IgG HRP-conjugated secondary antibody in blocking solution. Bands were visualized by chemiluminescent substrate kit. Anti-actin goat polyclonal antibody and Rabbit anti-goat IgG – HRP conjugated were used for equal loading confirmation. The density of bands was estimated as uncalibrated optical density using Image J software referenced to actin band (Burnette 1981).

Cell viability analysis

The viability of AD-MSCs was tested in different passages by adding equal volumes of the cell suspension and 0.4% trypan blue dye and then counting the clear and blue stained cells as alive and dead cells, respectively using a hemocytometer.

Quantitative real-time RT-PCR

Total RNA was extracted from testis samples using RNA easy Mini Kit (Invitrogen) according to the protocol. Reverse transcription was done

using (SMART_PCR cDNA synthesis kit, Clontech Inc., Palo Alto, CA). Quantitative RT-PCR was performed in duplicate in 25 µL mixture containing 1 µL template cDNA, SYBR Green PCR Master Mix (Applied Biosystems), 10 pmol of each Primer for CD44 forward, GATGGAGAAAGCTCTGAGCATC and reverse, CACCTTCTTCGACTGTTGAC, and for GAPDH forward, AACTTTGGC- ATTGTGGAAGG and reverse, GTCTTCTGGGTGGCAGTGAT. Reactions were run in I Cycler iQ (Bio-Rad). The results were normalized to GAPDH mRNA level.

Measurement of pro-inflammatory cytokines

The level of serum TNF-α, IL-6, and IL-10 were measured using an ELISA kit for each one (Ray Biotech, Georgia) according to the manufacturer's instructions.

Histological preparation and testis histopathology score

For histological and histopathological examinations, pieces of the testis were fixed in 10% neutral buffered formalin pH 7.2. Paraffin sections of 5 micrometers in thickness were prepared and then stained with haematoxylin and eosin stains. Seven sections of different animals were examined for each animal group. Five testis injury parameters (tissue vacuolization, cytoplasmic color fading, nuclear condensation, nuclear fragmentation and erythrocyte-stasis) were considered to score the testis injury according to Heijnen`s method (2003).

Statistical analysis

All results are expressed as the mean ± S. E. of at least three independent experiments. Statistical significance of differences was analyzed with one-way analysis of variance followed by the Student Newman-Keuls T-test. Results were considered statistically significant when $P<0.05$.

RESULTS

Morphological characterization of the cultured AD-MSCs

The segregated AD-MSCs began to stick to the plastic substrate of cell culture flasks inconsistently accomplishing various shapes three days after essential culture.

On day five, the cells turned out to be increasingly packed, the cells multiplied quickly and arrived at 90% confluency of primary culture. The cells were passaged about two times in 7^{th} and 9^{th} days. The cells from passage number three on days 9 were of homologous formed phenotype (Figure 1 a&b).

Flow cytometry analysis for AD-MSCs markers

Flow cytometry examination demonstrated that AD-MSCs at passage 3 were positive for CD105 (95.7±5%) and CD44 (85.7±3%) but conversely, negative for CD 14 (1.6±4%) (Figure 2).

Immunoblotting analysis for AD- MSCs markers

Western blot detection of AD-MSCs markers at passage 3 revealed the detection of CD105 (95 kDa) and CD44 (200 kDa). Densitometric analysis of the immune-reactive bands demonstrated that CD 105 and CD 44 was 4.4 & 2.8 fold, respectively. While there was no reaction for CD14 (54 kDa) (Figure 3a,b).

Cell viability of AD-MSCs

AD-MSCs viability was estimated by trypan blue-exclusion method. The experiment began with cell density at passage zero 112.9 $x10^3$ ±12.58 cells/ml. The cell viability at P1, P2 and P3 of AD-MSCs reached (538.5 $x10^3$ ± 84.70, 593.9 $x10^3$±67.53 & 616.3$x10^3$±57.51) respectively, compared with passage zero (Figure 4).

The results of HCl group were similar to that of the control group accordingly, the only results of the control group were shown**.**

AD-MSCs regulate P53 in the testis of rats administrated with As III L &H doses

Administration of As III in L &H doses elevates the level of p53 up to 2.4 and 3.5 fold, respectively versus those of control (Figure 5a) as determined from densitometric estimation (Figure 5b). Low and high doses of As III treatment was sufficient for expanding p53 to practically a large portion of the degree of control. Whereas As III L & H + AD-MSCs co-administration decrease the level of p53 (Figure 5a,b). So the impact of AD-MSCs displayed right around 1 and 1.3 folds alleviation in p53 level compared with the As III L&H alone, respectively.

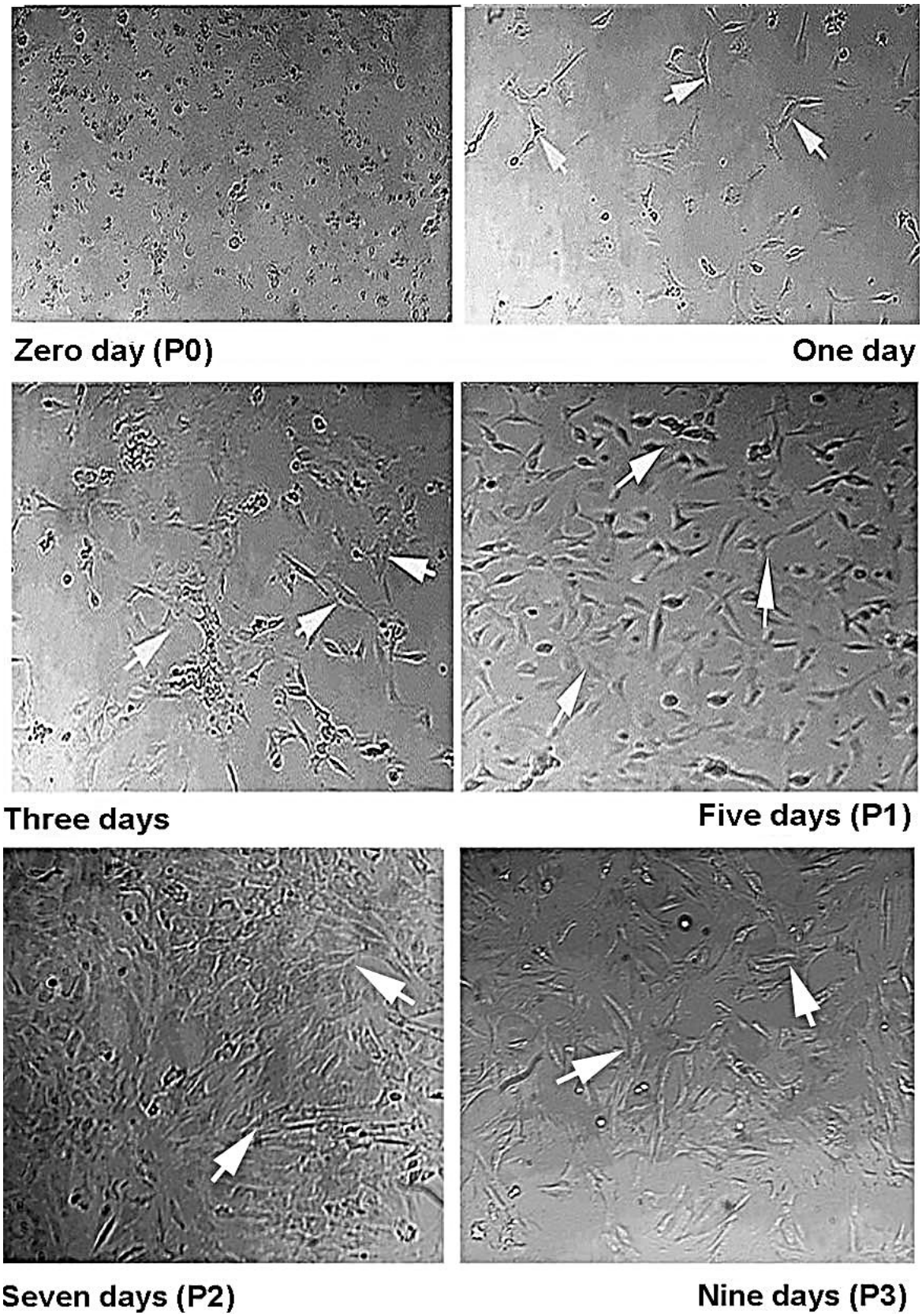

Figure 1. Morphology of AD-MSCs detected by phase contrast microscopy. On zero-day of primary culture, the AD-MSCs are spherical, on one day AD-MSCs adhere to the plastic surface and appear with variable sizes and shapes (white arrow). On day 5 some cells appear spindle shape, the cells forming small colonies and reach about 85-90% confluency of primary culture. 2^{nd}&3^{rd} passaged of AD-MSCs appear homogenous in shape. Most of the cultured cells have fibroblastic appearance (x 400).

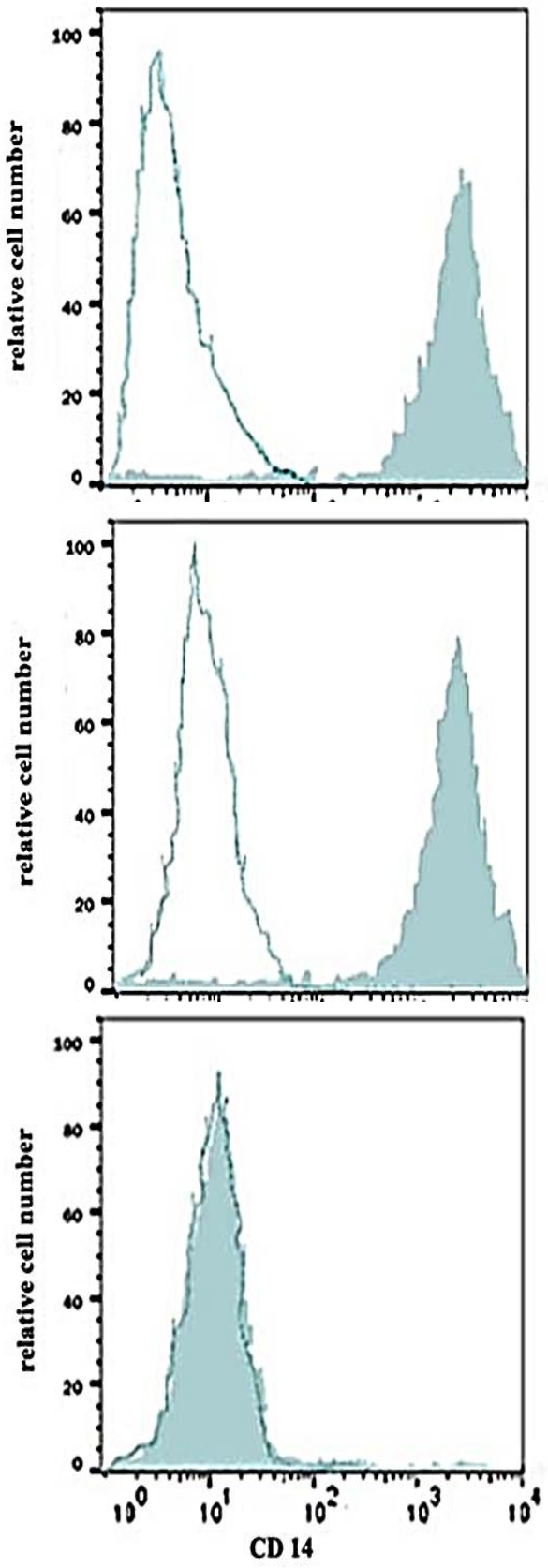

Figure 2. Flow cytometry analysis of the AD-MSCs at passage 3. More than 98% of the cells are positive in the expression of superficial characteristics of stem cells CD105 and Cd44 but they are negative for CD14.

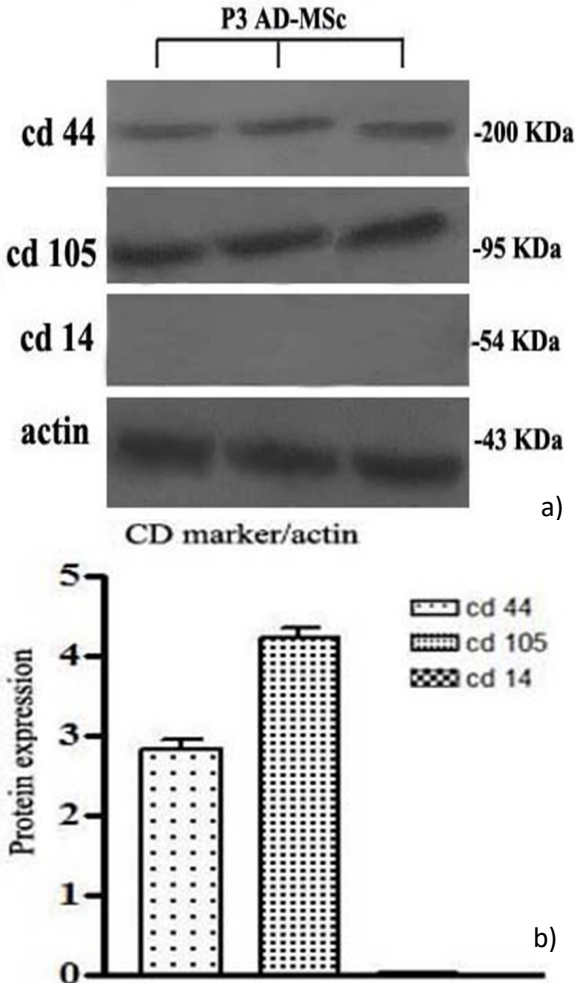

Figure 3. Immunoblotting analysis of AD-MSCs at passage 3 showing CD105 & CD 44 (95 kDa & 200 kDa) are significantly positive whereas CD14 (54 kD) is significantly negative (a). The proteins levels were quantitatively determined as a fold protein/actin. Data are presented as mean ± S.E. from at least three independent experiments (b).

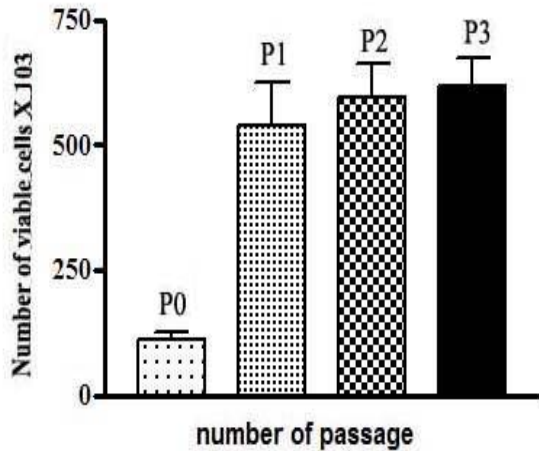

Figure 4. Mean viable AD-MSCs cells ± SE at 1 to 9 days of culture (experiment began with cell density 112.9 X 10^3 cells/ml).

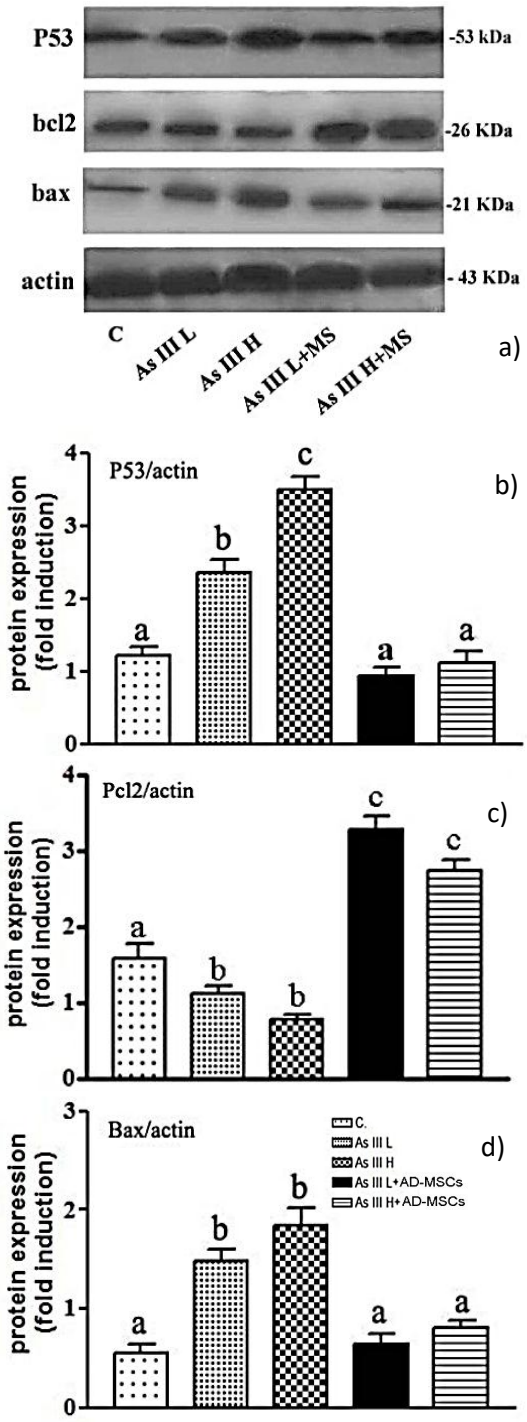

Figure 5. AD-MSCs stimulate the alleviation of P53 & Bax and BCL2 to increase in the testis of As III L& H treated rats. Testicular tissues were lysed and processed to western blot detection of p53, bcl2 & Bax as described in methods. Equal protein loading was verified with anti-actin (a). Immunoblots were quantified denistometricaly for the effect of exposure to As III L & H and different treatments on P53, BCL2 & Bax protein levels as fold induction of protein/actin ratio after normalization to the control level. Values in the same column with unlike superscript letters are significantly different at $P < 0.05$. Data are representing as the mean ±S.E. from at least three independent experiments (b, c, d).

AD-MSCs effect on apoptotic markers in the testis of As III L&H doses treated rats

Bcl2 as anti-apoptotic protein was found to be decreased by As III L &H treatment whereas, a noticeable increase in the level of Bax as pro-apoptotic protein after As III L &H treatment was detected (Figure 5a). Bcl2 level decreased almost 1.1 folds in As III L treatment and almost 0.7 folds in As III H versus to the control (Figure 5c). AD-MSCs co-treatment induced a sharp increase in Bcl2 level compared to As III L & H alone (Figure 5c). Bax level increased almost 1.5 folds in As III L treatment and around 1.8 folds in As III H versus to the control. In contrast to this, AD-MSCs decreased the level of Bax by 0.6 and 0.8 folds versus to As III L & H treatments (Figure 5d).

qRT-PCR for CD44 gene expression in testicular tissue

To evaluate the settlement of AD-MSCs in the testicular tissues, samples were taken for qRT-PCR analysis from all groups of the experiment for quantification of CD44 mRNA expression. The only samples that give a detectable signal for CD 44 mRNA were those received AD-MSCs transplantation, (As III L&H + AD-MSCs) experimental groups. The level of CD44 mRNA expression reached almost 8.3 and 5.5 folds for low and high As III doses transplanted with AD-MSCs (Figure 6). But no expression of CD44 was noticed in control, As III L & H treated rats. This observation confirms that AD-MSCs settled in testicular tissue of animals subjected to AD-MSCs transplantation (Figure 6).

Effect of AD-MSCs on pro-inflammatory cytokines

The Serum TNF-α, IL-6, and IL-10 in the blood of different treatment groups are shown in Figure 7a,b,c). As III L & H -treated rats showed a significant increase in the level of TNF-α (136.5% ±4.5 & 205.8% ±3.8) respectively. In addition to this, down regulation of IL-6, and IL-10 levels were obtained (51.05 %±2.7 & 68.6 %±2.9; 62.3%± 2.1& 82.2%± 1.7) respectively, versus control rats. The injection of AD-MSCs showed a significant reduction TNF-α levels in low and high doses (53.1 %±3.1 &47.9 %±3.8), respectively as well as an increase in IL-6, and IL-10 levels by (130.5 % ±4.4 & 163.1% ±3.7; 101.9

% ± 2.6 & 236.3% ±5.4) versos As III L & H - treated rats. The protective effect of AD-MSCs appeared clearly as a reduction of the level of TNF-α and increase of IL-6, and IL-10 levels in low and high doses of arsenic.

Light microscopic examination:

Histological examination of normal testis of rats stained by H&E in the control group (Figure 8a) appeared as normal histological structure: the testicles seemed encompassed and covered with a fibrous capsule called the tunica albuginea. The seminiferous tubules showed up firmly stuffed and lined by spermatogenic epithelium which framed of spermatogonisis stages. The Sertoli cells are also present in the epithelium of the seminiferous tubule. The seminiferous tubules were ensheathed by storm cellar layer, interstitial cells located between the seminiferous tubules.

In As III L group, there were great changes in the testicular structure including loss of regular spermatogenic epithelium stages arrangements and several degenerative germ cells were scattered in an irregular manner forming degenerative space. The cytoplasm is vacuolated with faintly stained nuclei. The vacuolization of the affected seminiferous tubules indicates obvious signs of hydropic degeneration. Also, the reduction in the number of interstitial cells leaving wide interstitium and intertubular space. Karyolitic nuclei of many spermatoginic cells and alumina of seminiferous tubule with empty sperms appeared in Figure 8b,c.

Microscopic examination of As III H group revealed serious and severe mutilation of the vast majority of the seminiferous tubules with loss of their ordinary histological structure. The tubules were contracted, decayed, and totally without spermatogenic epithelium leaving huge spaced empty areas. While many spermatogenic cells had shrunken pyknotic nuclei and other tubules filled with congested blood vessels in the interstitium (Figure 8d,e). In As III L & H+ AD-MSCs groups remarkable normal features of the seminiferous tubules with repaired spermatogenic cells in the seminiferous tubules and the interstitial cell number are restored. Slight pyknotic nuclei throughout some spermategenic cells were detected beside little degeneration of spermatocytes of tubules was observed (Figure 8f,g). Testis histophathological score was assessed by Heijnen's score, As III H groups recorded highest scores than As III L, Experimental groups of AD-MSCs showed significant decreasing of the score compared with As III L & H groups (Figure 8h).

DISCUSSION

As III is toxic and induce apoptosis and actuated oxidative stress in rat hepatocellular carcinoma cells and testis. Furthermore, As III will obstruct the cell cycle at G2/M stage. It can likewise actuate apoptosis to cells at different stages of the cell cycle (Higuchi et al., 2013; Zubair et al., 2017 and Boregowda et al., 2018).

In the present investigation, there was a noteworthy increment in testicular P53 and Bax levels and abatement of BCl2 level after As III L &H administration. The current result was in concordance with different data confirming the liver and kidney harm actuated by As III (Charles 2014 and Mehrzadi et al., 2018). Sodium arsenite-initiated oxidative stress, change in p53 pathway and apoptosis of testicles were observed in rat (Banik et al., 2014; Boregowda et al., 2018). Also, Prockop (2012) reported that Bax protein is an apoptotic factor and it is actuated in states of oxidative stress instigated apoptosis.

Numerous reports have examined the effect of AD-MSCs differentiation to myocytes, hepatocytes, neural cells and even epithelial cells of the lung, kidney and skin. (Komori et al., 2005; El Kasaby et al, 2018). The remedial capability of AD-MSCs depends on its antifibrotic, regenerative, and reparative properties; it could improve the harmed tissues (Lodi et al., 2011) and different illnesses including wound healing, neurological and lung infections, diabetes and cystic fibrosis (Fazeli et al., 2018; Sherif et al., 2018). The present result clarified the role of treatment with AD-MSCs in inhibition of the level of P53 and Bax in the testis of ASIII L&H treated mice, where AD-MSCs injection significantly stimulated the levels of BCl2 on These data correspond with previous reports in the role of MSCs with antioxidative and as anti-inflammatory capabilities (Burova et al., 2013).

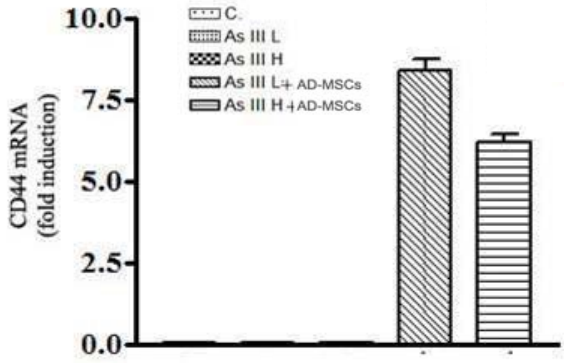

Figure 6. The mRNA of CD44 was quantified in testicular tissues by qRT-PCR in control and different treatments. CD44 is positively reacted in AD-MSCs co-administration groups, values were normalized by GAPDH corresponding level. Presented Data are shown by mean ±S.E. from three independent experiments.

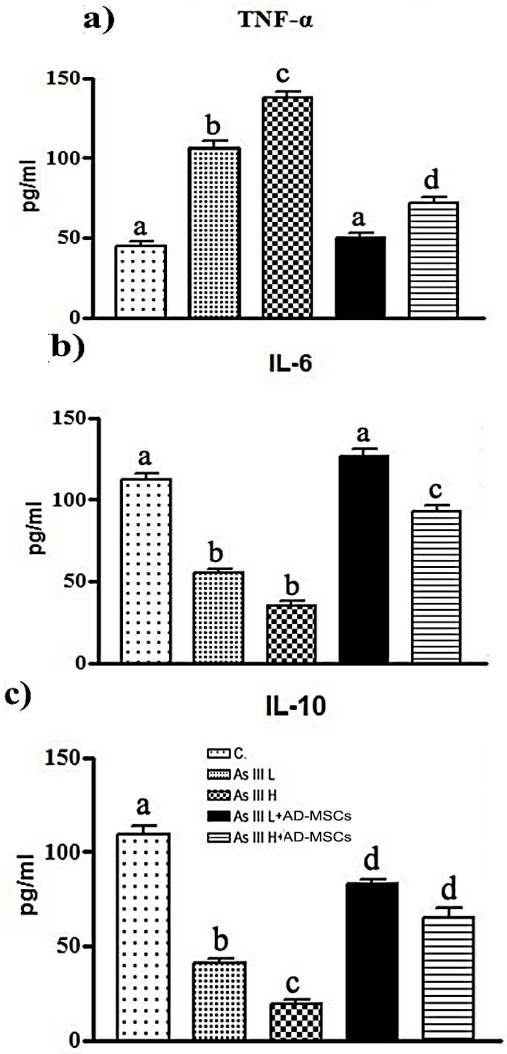

Figure 7. Showing the effect of As III L & H and its combination with D-MSCs on the levels of pro-inflammatory cytokines TNF-α, IL-6, and IL-10 determined in serum (a, b & c). Data are representing as the mean ±S.E. Unlike superscript letters in the same column are significantly different at $P < 0.05$.

For instance, Sheri et al., 2015 and Wang et al., 2017 recommended that the BM-MSCs induced decreases in caspase-3, and Bax levels in rat renal tissue treated with cytotoxic drug as cisplatin or heavy metal as cadmium. Furthermore, MSCs have been found to improve the testicular tissue toxicity due to their antioxidative defensive mechanism against oxidative stress (Hassan and Alam., 2014 and Roushandeh et al., 2017).

Some investigations have indicated that a few cells separated from mesenchymal stem cells could communicate germ cells explicit markers (Mazaheri et al., 2011). The present result shows that AD-MSCs at passage 3 revealed positive reactions for CD105 and CD44 while the protein expression level for CD14 gave a negative reaction.

Khurana et al., (2013) work revealed that negative expression for the antigens CD45 and CD31 in the MSC, despite CD44 and CD90 were expressed observably. In the present study, the gene expression analysis by qRT-PCR revealed the presence of AD-MSCs in the testis of rats by identifying the expression of CD44 positive cells in testis. The total count of CD44 positive cells showed a significant increase in stem cells-treated As III L&H groups as compared to As III L&H groups alone which lacked CD44.

A noteworthy increment of TNF-α levels was appeared in As III groups when compared to control. A comparable finding was seen by Sherif et al., (2018), affirming the toxicity of arsenic in several organs in the male rat. On the other hand, expanded degrees of oxidative stress due to exposure to arsenic were reported by others (De Vizcaya-Ruiz et al., 2009). The Oxidative stress stimulates cascades of inflammatory mediators including TNF-α causing cytotoxic effects and triggering of apoptosis (Liu et al., 2014 and Adikwu et al., 2014).

The present investigation indicated that treatment injured rat with AD-MSCs ameliorated the increased level of TNF-α protein compared to As III L &H groups. In contrast, AD- MSC administration increased the levels of IL-10 and IL- 6 in As III L& H –treated rats.

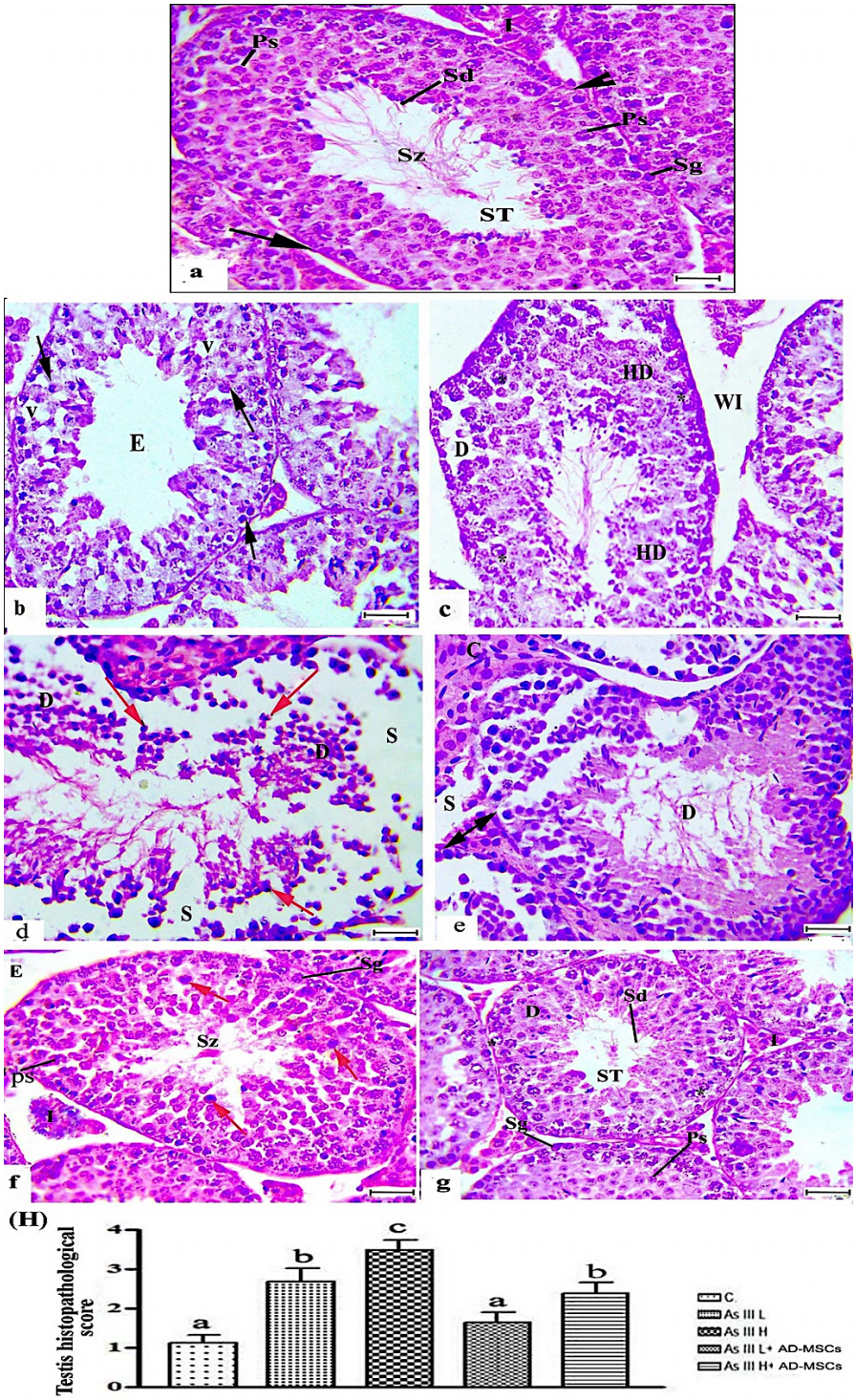

Figure 8. Light microscopic examination of testis sections of rats in the control and different treatments: Showing, normal structure of the testis and its adjacent seminiferous tubules (ST), boundary tissue (arrows), spermatogonia (Sg), primary spermatocytes (PS), spermatids (Sd), and spermatozoa (SZ) in each tubule, interstitial cells (I) and Sertoli cells (SC) are observed (arrows head) (a). In As III L group, several degenerative germ cells (D), spermatogenic cells had karyolytic nuclei (black arrow), vacuolization (V), hydropic degeneration (HD) and seminiferous tubule Lumina with empty sperms (E) (b &c). In As III H group, severs mutilation and shrunken of the vast majority of the seminiferous tubules leave huge space empty area (E), pyknotic nuclei of many spermatogenic cells (red arrow) and between tubules filled with congestion (C) were observed (d & e). As III L & H+ AD-MSCs groups: Showing Repair and improvement in the structure of the cell of the seminiferous tubules (ST) in two groups. But few pyknotic nuclei were detected in some spermategenic cells (red arrow) in As III L+ AD-MSCs group. However in As III H+ AD-MSCs group moderate degeneration (D) of spermatocytes of tubules was stilled appeared (f & g) (H&E, Bar = 50 µm). In testis histophathological score, Data are mean ± S.E. from two independent experiments. Values in the same column with unlike superscript signs are significantly different (P<0.05) (h).

Related studies have shown that upon MSC migration to the injured place, it becomes activated and secrete growth factors and cytokines such as IL-6 and platelet-inferred development factor (EL-Far et al., 2012; El Kasaby et al. , 2018). It may likewise up regulate IL-10 cytokine in the unhealthy organ (Metwally et al., 2017). A few investigations have indicated that these cytokines had a regulatory effect in the relocation of BM-MSCs and repairing harmed tissue (Kholodenko et al., 2013). Also, cytokines can manage cell processes, for example, chemotaxis, cell expansion, signaling of cell and angiogenesis in the harmed area (Asahara et al., 1999).

In the present study, light microscopic assessment of testicles in rats of the control group showed ordinary develops seminiferous tubules. Severe histopathological change of testis result by oral administration of As III in rats as in As III L & H groups testicular tissue has many damages compared to the control. These damages include congestion between tubules, hydropic degeneration in germ cells, vacuolated cytoplasm and Karyolitic nuclei of many spermatoginic cells. It was found that sever declined spermatogenic cells due to an essential impact on the cell-to-cell intersections among Sertoli and germ cells lead to germ cell apoptosis (Blanco-Rodríguez and Martínez-García 1998). Furthermore, exhaustion of germ cells due to oxidative stress is in concurrence with other reports (Aggarwal et al., 2007; Bal et al .,2012). In the present result, AD- MSCs improved the harmful effect of As III L & H in testicular tissues. However, a few investigations have indicated the gainful effect of MSCs in testicular injuries incited by various agents in different organs and animals (Cakici et al., 2015; Elawady et al., 2016). (Lue et al., 2007) suggested, the blood-testis barrier may help the MSCs to preserve from immunologic responses. The result of the current study revealed that the injection of AD-MSCs into rats caudle vein could help to repair severe damage in testis induced by arsenic.

CONCLUSION

This work was done to show the effective role of AD- MSCs against As III L &H induced testicular damage. AD-MSCs transplantation evoked changes in pro-inflammatory cytokines levels and expanded apoptosis boundary levels in rat testis. Also, the present results demonstrate the improvement effect of AD-MSCs on the histological structure of testis after exposure to As III L & H.

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CONFLICT OF INTEREST

Authors declare that they have no conflicts of interest.

FUDING

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Acknowledgements*:* In this section, the authors may acknowledge (briefly) their support staff.

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- If a cropped image is included in the main text of a paper (e.g. a few lanes of a gel), display the full original image, including the appropriate controls, the molecular size ladder and/or the scale as relevant, as a single figure in a Supplementary Material file to facilitate peer-review and for subsequent online publication.
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