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Evaluation of Mesenchymal Stem Cells and Vitamin E in Treatment of Infertile Male Albino Rats

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Abstract

The present study describes the effect of mesenchymal stem cells and vitamin E on infertility of adult male rats. Induction of infertility by oral administration of cadmium chloride ($cdcl_2$) for 30 days. Induction of infertility caused a reduction in body weight as well as in genital sex organs weight. The epididymal sperm counts and their motility were significantly decreased following $cdcl_2$ administration, while significantly increased in fructose in semen. A reduction in serum levels of luteinizing hormone (LH) and testosterone, and an elevation in serum follicle stimulating hormone (FSH) and prolactin (PRL) were also noticed in infertile rats. Significant increases in malondialdehyde (MDA), nitric oxide (NO) and catalase (CAT) levels were observed in testicular homogenate of infertile rats. Decreased in reduced glutathione (GSH) content, Superoxide dismutase (SOD) and total antioxidant were also found in induced infertility rats. Results showed that stem cells significantly increased of SOD in testes, LH and total antioxidant in serum, fructose in semen and fix all disruption caused by induction of infertility. Results demonstrated the beneficial influences of stem cells and vitamin E in reducing the harmful effects of $cdcl_2$ while vitamin E was less effective than stem cells. DNA fragmentation of testes from experimental animals was examined. Liver functions, kidney function, were estimated.

Keywords: *Infertility; $Cdcl_2$; mesenchymal stem cells; vitamin E; antioxidant; SOD ; NO CAT ; GSH ; MDA ; LH ; FSH ; PRL ; testis and DNA fragmentation.*

Introduction

Approximately 50% of human infertility is attributable to male defects, 70–90% of which arises from impaired spermatogenesis **(Greenberg *et al.,* 1978 and Sigman *et al.,* 1997).** Environmental factors have been suggested to play a role in animal infertility **(Hovatta *et al.,* 1998)**. There is epidemiological evidence that exposure to industrial metal aerosols may be detrimental to the male reproductive system and that the effects are not reversible by short periods of non-exposure **(Bonde, 1990)**. Acute exposure to cadmium may occur mainly at the work place by inhalation of fumes and dusts and occasionally by ingestion **(Järup, 2002)**. The general population may be chronically exposed to cadmium by contaminated food or water intake and by inhalation **(Satarug *et al.,* 2003)**. This heavy metal has the potential to affect reproduction and development in many different ways, and at every stage of reproductive process **(Thompson & Bannigan, 2008).** Testes seem to be greatly affected by cadmium since it has a unique vascular system **(Santos *et al.,* 2004 & 2005 and Hassanin & Safwat, 2014)**.

Cadmium is listed among the hazardous chemicals because it can enter the food chain (**Schenkel, 1988**) and it has a long biological half-life (about 30 years) in humans (**Cotzias *et al.,* 1961; Nordberg & Kjellstrom, 1979)** and its toxicity is dependent on the route, dose and duration of exposure **(Goering *et al.,* 1987; Goyer *et al.,* 1995 and Satarug *et al.,* 2003)**. After absorption, cadmium is transported to the liver, bound to albumin **(Nordberg *et al.,* 1992)**, where it induces the synthesis of metallothionein (MT), and a class of small cysteine-rich heavy metal binding proteins **(Sauer *et al.,* 1997 and El-Ashmawy & Youssef, 1999)**. Once absorbed, cadmium is rapidly cleared from the blood and concentrates in various tissues. Chronic exposure to inorganic cadmium results in accumulation of the metal mainly in the liver and kidneys, as well as in other tissues and organs causing many metabolic and histological changes, membrane damage, altered gene expression and apoptosis **(Stohs & Bagchi, 1995; Stohs *et al.,* 2001 and Waisberg *et al.,* 2003).**

Vitamin E is a family of lipid-soluble vitamins, of which α-tocopherol is the most potent **(Beyer, 1994)**. In 1922,

Evans & Bishop discovered vitamin E a micronutrient essential for reproduction in rats. Vitamin E subsequently proved to be effective in preventing lipid peroxidation and other radical-driven oxidative events **(Tappel, 1962; Burton & Ingold, 1986 and Esterbauer, 1991)**. It is well known that vitamins E is antioxidative agents that act synergistically as potent scavengers of free radicals and terminators of free-radical chain reactions (**McCay, 1985; Niki, 1987 and Burton & Ingold, 1989**).

Stem cells are capable of self-renewal and can differentiate into specialized cell types **(Bruder *et al.,* 1997; Chopp *et al.,* 2000; Sato *et al.,* 2005 and Abdel Aziz *et al.,* 2007)**. Bone marrow (BM) contains at least two types of stem cells, hematopoietic and non-hematopoietic stem cells, usually called mesenchymal stem cells (MSCs). MSCs are of great interest because they are easily isolated from a small aspirate of BM and readily generate single-cell-derived colonies, which can be expanded through as many as 50 population doublings in about 10 weeks **(Abdel Aziz *et al.,* 2007)**.

Anderson *et al.* (2001) reported that stem cells are potentials for treating different diseases inducing diabetes, liver, heart diseases and infertility.

Materials and Methods

Experimental animals

The experimental animals used in this study were adult male albino rats, weighing (250 ± 25 g). The animals were obtained from the animal house of the National Organization for Drug Control and Research (NODCAR), Egypt. The animals were housed in plastic cages allowed to adjust to the new environment for two weeks before starting the experiment for adaptation. The rats were kept at 25 ± 2°C and 55 ± 5% humidity with 12 h light/ dark cycle *adlibitum* with a basal pelleted diet and water. Anesthetic procedures and handling with animals were approved by and complied with the ethical guidelines of Medical Ethical Committee of the National Research Centre in Egypt **(Approval number: 10031)**.

Induction of Infertility

Two week after acclimatization, 24 rats for control groups and 48 rats were rendered infertility by daily oral administration of $cdcl_2$ 5 mg/kg body wt/day 1/15 LD_{50} **El-Demerdash *et al.* (2004)** for 30 days. At the end of 30 days, blood samples were collected and processed for testosterone, LH, sperm motility and sperm count. The rats which achieved infertility as represented by significant decrease in testosterone, LH, sperm motility and sperm count compared to control groups.

Experimental design

Rats were divided as follow:

1-Control group: animals receiving a daily oral administration of distilled water for 30 days (n=12).

2- Vitamin E control group: animals received a daily oral administration of vitamin E dissolved in olive oil solution in a dose of (400 mg/kg body weight/day) for 30 successive days (n=12).

3- Infertile group: animals receiving a daily oral administration of $cdcl_2$ (5 mg/kg body wt/day) for 30 days (n=48). $Cdcl_2$ was dissolved in water and this group divided into 3 subgroups.

a-Stem cells-treated group: After 24 hours from the last dose of $cdcl_2$, animals were treated with a single intratesticular injection of rat bone marrow mesenchymal stem cells containing 1×10^6 cells then leave for 30 days (n=12).

b- Vitamin E-treated group: After 24 hours from the last dose of $cdcl_2$, animals were orally administered of vitamin E dissolved in olive oil solution (400 mg/kg body wt/day) for 30 successive days (n=12).

c- Withdrawal period group (W.P): After 24 hours from the last dose of $cdcl_2$, the animals leave for 30 days without any injection of $cdcl_2$ (n=12).

Patches of 10 rats from each group were taken for blood collection and scarified.

Body weight measurement

Body weight was recorded daily beginning on zero time (the time prior to treatment) and continued until sacrifice. The body weight was averaged for each two week until the end of the treatment.

Blood collection

Blood samples were collected from retro-orbital plexus into serum separation tube after 30 days **(Schermare, 1967).** The blood was allowed to clot at room temperature for 30 minutes. Serum was then separated by centrifugation at 3000 revolution per minute (rpm) for 10 minutes at 4°C. The separated serum was collected and divided into aliquots, then stored at -20°C for further determinations of sex hormones, liver function, kidney function and total antioxidant capacity.

Tissue preparation

The rats were sacrificed by decapitation after exposing them to mild ethyl ether anesthesia **(Parmer & Kar, 2009)**. Pair of testes, epididymis, seminal vesicles, prostate gland as well as liver, kidneys and spleen was removed and cleared from adhering tissue, washed in ice-cold 1.15%KCL, plot dried with filter paper and weighted. The right caudal epididimus was used for sperm motility estimation, while the left one was used for sperm count determination. The right testis was kept in alkaline phosphate buffer and stored in -20°C for DNA fragmentation, while the left testis was stored at -20°C for oxidative stress and antioxidant parameters determination.

Epididymal spermatozoal examination

The epididymis content of each rat was obtained by cutting the tail of 933pididymis and examined according to the technique adopted by **Bearden & Fuquay (1997)** for the estimation of the following parameters:

a-Sperm count: The spermatozoa were counted by hemocytometer using the Improved Neubauer (Deep 1/10mm. LABART, Germany) chamber. Epididymis sperm was collected by slicing the epididymis in 5 mL phosphate buffered saline (pH 7.2) according to **Narayana *et al.* (2002)**. An aliquot of the epididymis sperm suspension was used for spermatozoa count using hemocytometer by **Linder *et al.* (1986)**.

b- Progressive motility: A small droplet of semen was added to one drop of sodium citrate water solution (2.9 %) on a slide. Several fields were examined, and classified into motile and none motile sperms, and the incidences of progressively motile sperms were estimated and recorded as percentage (%) of sperm motility by **Yokoi *et al.* (2003).**

Hormone assay: The levels of testosterone, Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH) and Prolactin (PRL) were determined in serum by using ELISA kits (diagnostic system laboratories INC.) supplied from (Monobind Inc., USA) according to the methods of **Ekins (1990); Knobil (1980); Pierce & Parsons (1981) and Knobil (1980)** respectively.

Oxidative stress parameters

Lipid peroxidation was quantified as malondialdehyde (MDA) according to the method described by **Uchiyama & Mihara (1978)** and nitric oxide (NO) content was assayed indirectly as nitrite concentration according to the method of **Miranda *et al.* (2001)**.

Antioxidant parameters

The content of reduced glutathione (GSH) was determined by using the method of **Van Dooran *et al.* (1978)**, Superoxide dismutase (SOD) activity was carried out according to the method of **Nandi & Chatterjee (1988)**. The catalase (CAT) activity was estimated at 240 nm according to **(Aebi, 1984).** The total antioxidative capacity was performed by **Koracevic *et al.* (2001).**

Liver and kidney functions

Liver functions including serum ALT, AST, Alkaline phosphatase (ALP), albumin and total protein were measured enzymatically in serum according to **(Reitman & Frankel, 1957** for both ALT and AST**; George & Robert, 1975**; **Doumas, 1971 and Koller, 1984** respectively**).** While kidney functions (urea and creatinine) were measured enzymatically in serum as described by (**Fawcett & Scott, 1960 and Henery et al., 1974** respectively**).**

Preparation of BM-derived MSC

Bone marrow was harvested by flushing the tibiae and femurs of six week old male white albino rats with Dulbecco's modified Eagle's medium (DMEM, GIBCO/BRL) supplemented with 10% fetal bovine serum (GIBCO/BRL). Nucleated cells were isolated with a density gradient (Ficoll-Paque, Pharmacia) and resuspended in complete culture medium supplemented with 1% penicillin-streptomycin (GIBCO/BRL). The cells were incubated at 37°C in 5% humidified CO_2 for 12–14 days as primary culture or upon formation of large colonies. When large colonies developed (80-90 % confluence), the cultures were washed twice with phosphate-buffered saline (PBS) and the cells were trypsinized with 0.25% trypsin in 1 mM EDTA (GIBCO/BRL) for 5 min at 37°C. After centrifugation, the cells were resuspended with serum-supplemented medium and incubated in 50-cm^2 culture flask (Falcon). The resulting cultures were referred to as first-passage cultures according to **Alhadlaq & Mao (2004)**. MSCs in culture were characterized by their adhesiveness and fusiform shape as by **Rochefort *et al.* (2005)**.

DNA fragmentation

DNA fragmentation was carried out according to the method of **Singh *et al.,* (1988).**

Statistical analysis

The quantitative data of continuous variables were expressed as mean ± S.D. Significance of mean values of different parameters between the groups were analyzed using two way analysis of variance (ANOVA). LSD or James-Howell multiple comparison post hoc tests were performed to evaluate the significance of difference in means between various treatments groups according to the test for homogeneity. Values were presented as means ± SEM and a P value < 0.05 were considered significant.

All analyses were performed using SPSS 18 for Windows (SPSS Inc., Chicago, USA).

Results

1- Body weight

Figure (1) illustrates that, the change in body weight of $cdcl_2$ treated animals were similar to that of control animals from zero time to four weeks of treatment, after that there were decreases in the body weight gain until the end of the time treatment schedule when compared with controls. Table (1) shows that rats received $cdcl_2$ demonstrated a significant ($p < 0.05$) decrease in body weight gain after 4 weeks of treatment by (- 7.4%).

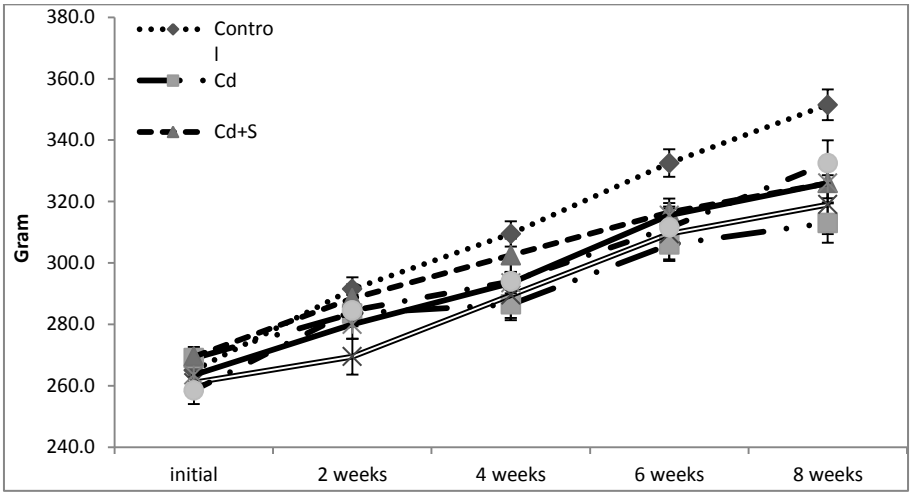


Figure (1): Effect of ($cdcl_2$, vitamin E and stem cells) on body weight of rats after initial, 2, 4, 6 and 8 weeks of administration and after withdrawal period (W.P). Values given are Mean ± S.E. (n=10).

- Groups sharing the same letters are not significantly different

Table (1): Effect of ($cdcl_2$ 5.0 mg/kg body wt, vitamin E 400 mg/kg body wt and stem cells in a volume of 0.5ml/rat containing 1×10^6 cells) on body weight of rats after initial, 2, 4, 6 and 8 weeks of administration and after withdrawal period (W.P). The number of animals was 10 for each group. Values given are Mean ± S.D.

	Initial (Weeks/g)		2 (Weeks/g)		4 (Weeks/g)		6 (Weeks/g)		8 (Weeks/g)	
	Mean ± SD	% Change	Mean ± SD	% Change	Mean ± SD	% Change	Mean ± SD	% Change	Mean ± SD	% Change
Control	265.0±12.02$^{(a)}$		291.5±12.26$^{(a)}$		309.5±13.01$^{(a)}$		332.5±14.19$^{(a)}$		351.5±15.82$^{(a)}$	
Cd	269.0±11.25$^{(a)}$	*1.5*	283.5±10.81$^{(a)}$	*- 2.7*	286.5±16.17$^{(b)}$	*- 7.4*	306.0±16.96$^{(b)}$	*- 8.0*	313.0±20.17$^{(b)}$	*- 11.0*
Cd + S	269.5±9.85$^{(a)}$	*1.7*	288.5±14.35$^{(a)}$	*- 1.0*	302.5±17.04$^{(ac)}$	*- 2.3*	316.5±14.15$^{(ab)}$	*- 4.8*	326.0±15.6$^{(bc)}$	*- 7.3*
Cd + E	261.0±13.50$^{(a)}$	*- 1.5*	269.5±18.48$^{(b)}$	*- 7.5*	289.5±23.39 $^{(b)}$	*- 6.5*	309.5±26.19$^{(b)}$	*- 6.9*	319.0±28.6$^{(bc)}$	*- 9.2*
E	263.5±15.28$^{(a)}$	*- 0.6*	280.0±14.91$^{(ab)}$	*- 3.9*	293.5±10.81 $^{(bc)}$	*- 5.2*	315.5±179 $^{(b)}$	*- 5.1*	326.0±19.69$^{(bc)}$	*- 7.3*
W	258.5±14.15$^{(a)}$	*- 2.5*	284.5±16.06$^{(a)}$	*- 2.4*	294.0±19.69 $^{(bc)}$	*- 5.0*	311.5±21.74$^{(b)}$	*- 6.3*	332.5±23.6$^{(c)}$	*- 5.4*
F Value	**1.16**		**2.75**		**2.49**		**2.58**		**4.08**	
P Value	**0.34**		**< 0.05**		**< 0.05**		**< 0.05**		**< 0.01**	

- One Way Analysis of Variance (ANOVA) was first applied.
- LSD test was used for multiple comparisons, P<0.05 is regarded as significant for homogenous variance equal assumed.
- James-Howell multiple comparison, P<0.05 is regarded as significant for homogenous variance not equal assumed.
- Groups sharing the same letters are not significantly different.

Treatment of rats with vitamin E and stem cells caused a gain in body weight. However, withdrawal period group, a slight improvement in the body weight.

2- Relative genital organs weight

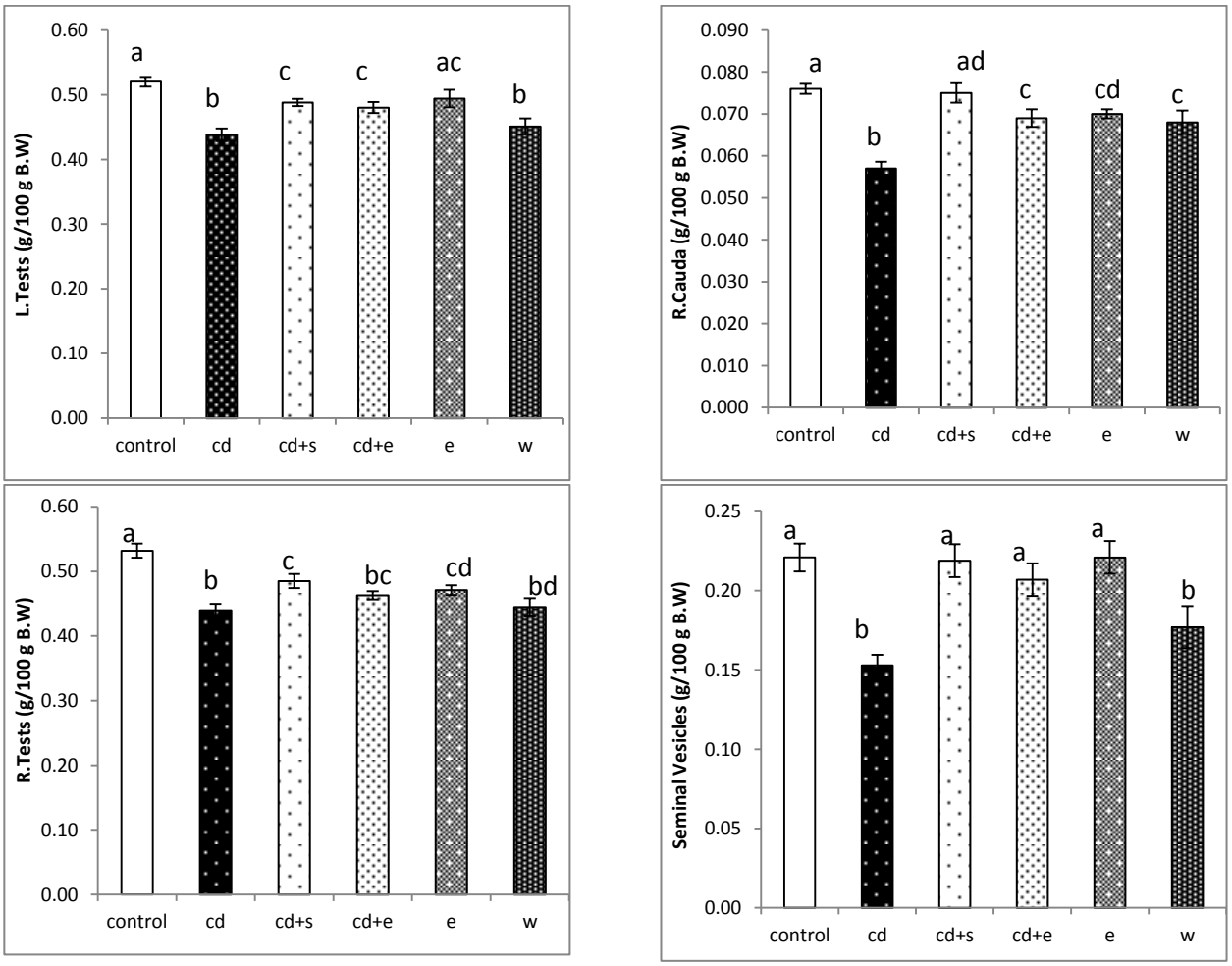
The result summarized in tables (2) and figure (2) revealed the occurrence of a gradual, sustained significant decrease ($p < 0.05$) in the relative weights of left and right testis, epididimus, seminal vesicles and prostate gland after 30 days of infertility induced by daily oral administration of $cdcl_2$. Administration of $cdcl_2$ for 30 days caused (-7.7%, -13.2%, -12.5%, -4.9% and -6.7% respectively) compared with the control animals. Treatment with vitamin E failed to exert significant change in the weight of left testis, right testis and epididimus relative to control (-7.7%, -13.2% and -12.5% respectively) while vitamin E countered $cdcl_2$ mediated effects on seminal vesicles and prostate gland, the weight returned to control.

However, injection of stem cells to $cdcl_2$ challenged rat's improvement the weight of all organs. After discontinuation of $cdcl_2$, the weights of left and right testis, epididimus, seminal vesicles and prostate gland not retained near their control values (-13.5%, -15.1%, -12.5%, -18.2 and -25% respectively).

Table (2): Effect of ($cdcl_2$ 5.0 mg/kg body wt, vitamin E 400 mg/kg body wt and stem cells in a volume of 0.5ml/rat containing 1×10^6 cells) on the relative weight (g/100g body weight) of genital organs (left testis, right testis, epididimus, seminal vesicle and prostate gland) of rats and after withdrawal period (W.P.). The number of animals was 10 for each group. Values given are Mean ± S.D.

	Left testis (g/100g B.W)		Right testis (g/100g B.W)		Epididimus (g/100g B.W)		Seminal vesicles (g/100g B.W)		Prostate gland (g/100g B.W)	
	Mean ± SD	% Change	Mean ± SD	% Change	Mean ± SD	% Change	Mean ± SD	% Change	Mean ± SD	% Change
Control	**0.52±0.008**$^{(a)}$		**0.53±0.011**$^{(a)}$		**0.08±0.001**$^{(a)}$		**0.22±0.009**$^{(a)}$		**0.20±0.009**$^{(a)}$	
Cd	**0.44±0.01**$^{(b)}$	*- 15.4*	**0.44±0.01**$^{(b)}$	*- 17.0*	**0.06±0.002**$^{(b)}$	*- 25.0*	**0.15±0.007**$^{(b)}$	*- 31.8*	**0.11±0.003**$^{(b)}$	*- 45.0*
Cd + S	**0.49±0.006**$^{(c)}$	*- 5.8*	**0.49±0.011**$^{(c)}$	*- 7.5*	**0.08±0.002**$^{(ad)}$	*0.0*	**0.22±0.01**$^{(a)}$	*0.0*	**0.18±0.006**$^{(ac)}$	*- 10.0*
Cd + E	**0.48±0.009**$^{(c)}$	*- 7.7*	**0.46±0.006**$^{(bc)}$	*- 13.2*	**0.07±0.002** $^{(c)}$	*- 12.5*	**0.21±0.01**$^{(a)}$	*- 4.5*	**0.18±0.009**$^{(ac)}$	*- 10.0*
E	**0.49±0.014**$^{(ac)}$	*- 5.8*	**0.47±0.008**$^{(cd)}$	*- 11.3*	**0.07±0.001** $^{(cd)}$	*- 12.5*	**0.22±0.01** $^{(a)}$	*0.0*	**0.16±0.011**$^{(cd)}$	*- 20.0*
W	**0.45±0.012**$^{(b)}$	*- 13.5*	**0.45±0.013**$^{(bd)}$	*- 15.1*	**0.07±0.003** $^{(c)}$	*- 12.5*	**0.18±0.013**$^{(b)}$	*- 18.2*	**0.15±0.009**$^{(d)}$	*- 25.0*
F Value	**8.81**		**10.87**		**11.95**		**7.71**		**14.17**	
P Value	**< 0.0001**		**< 0.0001**		**< 0.0001**		**< 0.0001**		**< 0.0001**	

- One Way Analysis of Variance (ANOVA) was first applied.
- LSD test was used for multiple comparisons, P<0.05 is regarded as significant for homogenous variance equal assumed.
- James-Howell multiple comparison, P<0.05 is regarded as significant for homogenous variance not equal assumed.
- Groups sharing the same letters are not significantly different



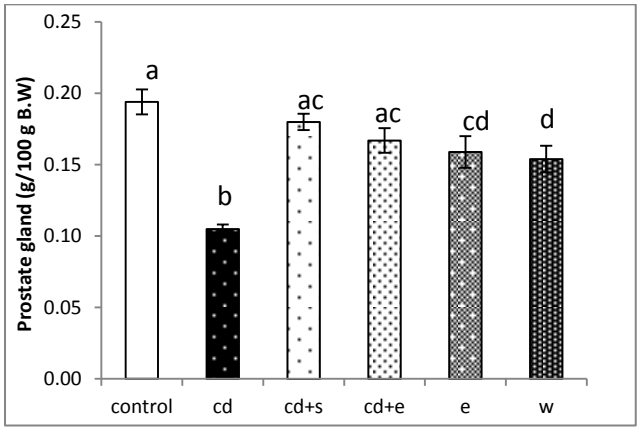


Figure (2) Effect of ($cdcl_2$, vitamin E and stem cells) on the genital organs (left testis, right testis, epididimus, seminal vesicle and prostate gland) of rats and after withdrawal period (W.P). Values given are Mean ± S.E. (n=10)

- Groups sharing the same letters are not significantly different

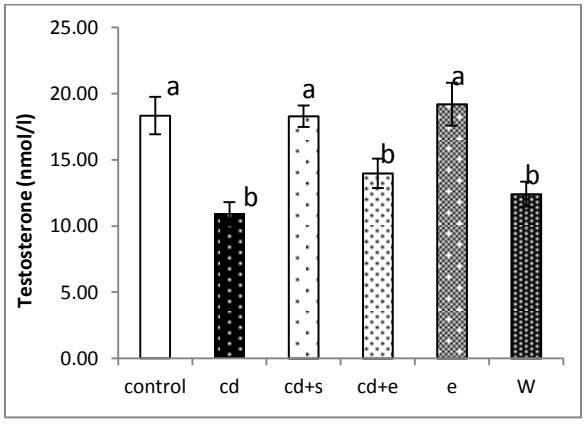
3- Sex hormones

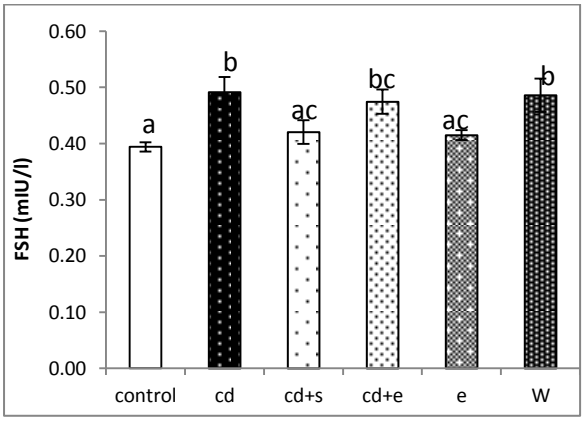
The data presented in table (3) and figure (3) show that administration of $cdcl_2$ for 30 days caused a notable decline ($P< 0.05$) in serum levels of testosterone and LH (-40.3% and -38.9 respectively), however, yielded a significant elevation in FSH and prolactin serum levels (25.6% and 22.2% respectively) relative to control values. The treatment of cd-exposed rats with stem cells reversed this change in LH (22.2 %), can fix all problems caused by $cdcl_2$ and retained to control group. In fact, no significant differences were observed when treated with vitamin E which caused by $cdcl_2$ in testosterone, FSH and prolactin (-23.8%, 20.5% and 18.1% respectively) except in LH, treatment occurred (-11.1%) when compared with control values. Also after cessation of $cdcl_2$, the levels of testosterone, LH, FSH and prolactin not retained near their control values (-32.3%, -33.3%, 25.6% and 17.1% respectively).

Table (3): Effect of ($cdcl_2$ 5.0 mg/kg body wt, vitamin E 400 mg/kg body wt and stem cells in a volume of 0.5ml/rat containing 1×10^6 cells) on serum levels of LH (mIU/ml), FSH (mIU/ml), Prolactin (mIU/l) and testosterone (nmol/l) and after withdrawal period (W.P.). The number of animals was 10 for each group. Values given are Mean ± S.D.

	Testosterone (nmol/l)		FSH (mIU/l)		LH (mIU/l)		Prolactin (mIU/l)	
	Mean ± SD	% Change	Mean ± SD	% Change	Mean ± SD	% Change	Mean ± SD	% Change
Control	**18.34±4.48$^{(a)}$**		**0.39±0.03$^{(a)}$**		**0.18±0.04$^{(a)}$**		**5.31±0.64$^{(a)}$**	
Cd	**10.94±2.74$^{(b)}$**	*-40.3*	**0.49±0.09$^{(b)}$**	*25.6*	**0.11±0.03$^{(b)}$**	*-38.9*	**6.49±0.88$^{(bc)}$**	*22.2*
Cd + S	**18.30±2.57$^{(a)}$**	*-0.2*	**0.42±0.07$^{(ac)}$**	*7.7*	**0.22±0.06$^{(c)}$**	*22.2*	**5.64±0.28$^{(ac)}$**	*6.2*
Cd + E	**13.98±3.55$^{(b)}$**	*-23.8*	**0.47±0.07$^{(bc)}$**	*20.5*	**0.16±0.03$^{(ad)}$**	*-11.1*	**6.27±0.31$^{(bd)}$**	*18.1*
E	**19.21±5.13$^{(a)}$**	*4.7*	**0.41±0.03$^{(ac)}$**	*5.1*	**0.14±0.03$^{(bd)}$**	*-22.2*	**5.93±0.24$^{(acd)}$**	*11.7*
W	**12.42±3.00$^{(b)}$**	*-32.3*	**0.49±0.09$^{(b)}$**	*25.6*	**0.12±0.02$^{(b)}$**	*-33.3*	**6.22±0.39$^{(bd)}$**	*17.1*
F Value	**9.1**		**3.94**		**12.38**		**7.43**	
P Value	**< 0.0001**		**< 0.01**		**< 0.0001**		**< 0.0001**	

- One Way Analysis of Variance (ANOVA) was first applied.
- LSD test was used for multiple comparisons, P<0.05 is regarded as significant for homogenous variance equal assumed.
- James-Howell multiple comparison, P<0.05 is regarded as significant for homogenous variance not equal assumed.
- Groups sharing the same letters are not significantly different.





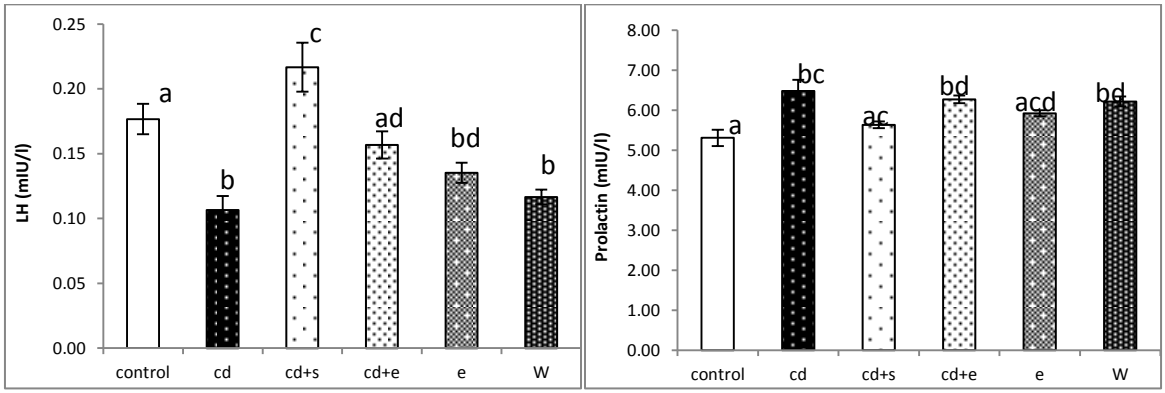


Figure (3) Effect of ($cdcl_2$, vitamin E and stem cells) on serum levels of LH (mIU/ml), FSH (mIU/ml), Prolactin (mIU/l) and testosterone (nmol/l) of rats and after withdrawal period (W.P). Values given are Mean ± S.E. (n=10)

- Groups sharing the same letters are not significantly different

4- Sperm parameters

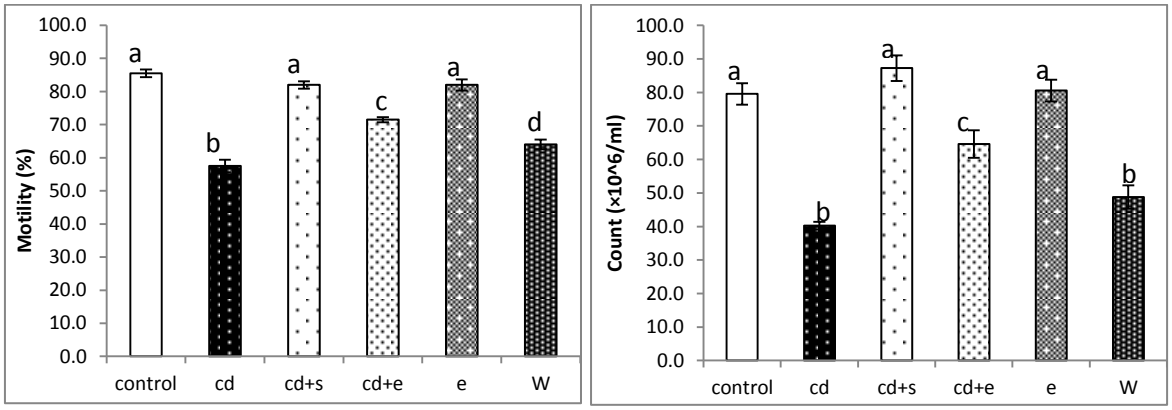
Figure (4) and table (4) clears that, rats received $cdcl_2$ for 30 days provoked a statistically significant (*P*< 0.05) decrease in sperm count and sperm motility (-32.7% and -49.5% respectively) relative to control rats. However, a significant increase in fructose content in semen relative to control group (32.0%) was detected after $cdcl_2$ exposure.

After treated with vitamin E it illustrate remarkable decrease in sperm count, motility and fructose (-16.4%, -18.8% and -44.7% respectively) in comparison with control groups. Meanwhile, treatments with MSCs significantly improve the sperm count and motility. In addition, fructose content decreased in comparison with control group (-40.9%). While at ending of $cdcl_2$ administration, the sperm motility and sperm count still significantly decreased to their control values (-25.1% and -38.7% respectively).

Table (4) Effect of ($cdcl_2$ 5.0 mg/kg body wt, vitamin E 400 mg/kg body wt and stem cells in a volume of 0.5ml/rat containing 1×10^6 cells) on the epididymal sperm count (10^6/ml), sperm motility (%) and fructose in semen (mg/dl) of rats and after withdrawal period (W.P.). The number of animals was 10 for each group. Values given are Mean ± S.D

	Motility (%)		Count (10^6/ml)		Fructose (mg/dl)	
	Mean ± SD	*%* Change	**Mean ± SD**	*%* Change	**Mean ± SD**	*%* Change
Control	**85.5±3.69**[(a)]		**79.6±10.08**[(a)]		**11.92±2.20**[(a)]	
Cd	**57.5±5.89**[(b)]	*-32.7*	**40.2±3.54**[(b)]	*-49.5*	**15.74±1.79**[(b)]	*32*
Cd + S	**82.0±3.50**[(a)]	*-4.1*	**87.3±12.05**[(a)]	*9.7*	**7.05±1.53**[(c)]	*-40.9*
Cd + E	**71.5±2.42**[(c)]	*-16.4*	**64.6±13.01**[(c)]	*-18.8*	**6.59±1.53**[(c)]	*-44.7*
E	**82.0±5.38** [(a)]	*-4.1*	**80.6±10.25**[(a)]	*1.3*	**11.50±2.52**[(a)]	*-3.5*
W	**64.0±4.60**[(d)]	*-25.1*	**48.8±11.00**[(b)]	*-38.7*	**13.1±2.68**[(a)]	*9.9*
F Value	**65.82**		**33.14**		**28.83**	
P Value	**< 0.0001**		**< 0.0001**		**< 0.0001**	

- One Way Analysis of Variance (ANOVA) was first applied.
- LSD test was used for multiple comparisons, P<0.05 is regarded as significant for homogenous variance equal assumed.
- James-Howell multiple comparison, P<0.05 is regarded as significant for homogenous variance not equal assumed.
- Groups sharing the same letters are not significantly different



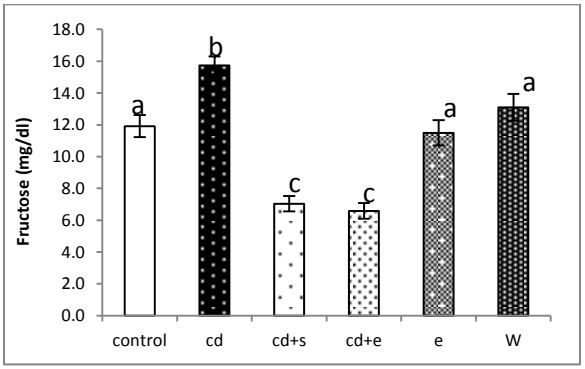


Figure (4) Effect of ($cdcl_2$, vitamin E and stem cells) on the epididymal sperm count (10^6/ml), sperm motility (%) and fructose in semen (mg/dl) of rats and after withdrawal period (W.P). Values given are Mean ± S.E. (n=10).

- Groups sharing the same letters are not significantly different

Table (5): Effect of ($cdcl_2$ 5.0 mg/kg body wt, vitamin E 400 mg/kg body wt and stem cells in a volume of 0.5ml/rat containing 1×10^6 cells) on oxidative stress parameters such as MDA (µmol/g tissue) and NO (µmol/g tissue) in testes and after withdrawal period (W.P.). The number of animals was 10 for each group. Values given are Mean ± S.D.

	MDA (µmol/g tissue)		NO (µmol/g tissue)	
	Mean ± SD	*%* Change	**Mean ± SD**	*%* Change
Control	**305.5±18.25**$^{(ad)}$		**0.36±0.06**$^{(a)}$	
Cd	**370.0±51.26**$^{(bc)}$	*21.1*	**0.41±0.04**$^{(bc)}$	*13.9*
Cd + S	**323.0±42.20**$^{(ac)}$	*5.7*	**0.35±0.05**$^{(a)}$	*-2.8*
Cd + E	**262.3±37.33** $^{(d)}$	*-14.1*	**0.36±0.05**$^{(a)}$	*0*
E	**289.5±51.25**$^{(ad)}$	*-5.2*	**0.37±0.05**$^{(ac)}$	*2.8*
W	**342.1±35.30**$^{(ac)}$	*12*	**0.38±0.06**$^{(ac)}$	*5.6*
F Value	**8.81**		**1.68**	
P Value	**< 0.0001**		**0.16**	

- One Way Analysis of Variance (ANOVA) was first applied.
- LSD test was used for multiple comparisons, P<0.05 is regarded as significant for homogenous variance equal assumed.
- James-Howell multiple comparison, P<0.05 is regarded as significant for homogenous variance not equal assumed.
- Groups sharing the same letters are not significantly different.

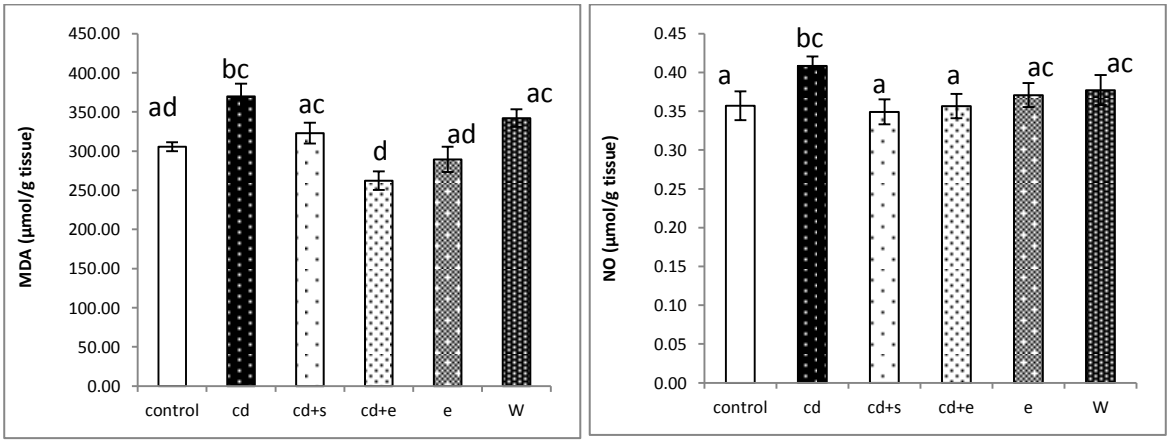


Figure (5) Effect of ($cdcl_2$, vitamin E and stem cells) on oxidative stress parameters such as MDA (µmol/g tissue) and NO (µmol/g tissue) in testes of rats and after withdrawal period (W.P). Values given are Mean ± S.E. (n=10).

- Groups sharing the same letters are not significantly different.

5- Oxidative stress parameters

As could be seen from Figure (5) and table (5), MDA and NO levels were impressive (P< 0.05) increased after 30 days from administration of cdcl2 as compared to controls (21.1 % and 13.9% respectively). There is a no significant difference in MDA and NO concentrations were found between stem cells group and control group. Whereas vitamin E partially reversed this change which displayed a significant decrease in MDA (-14.7%) While, a nonsignificant change occurred in NO when compared with control values. After discontinuation of $cdcl_2$, the levels of MDA and NO retained to their control values.

Table (6): Effect of ($cdcl_2$ 5.0 mg/kg body wt, vitamin E 400 mg/kg body wt and stem cells in a volume of 0.5ml/rat containing 1×10^6 cells) on antioxidant parameters such as GSH (µmol/g tissue), SOD (U/mg protein), CAT (U/mg protein) and total antioxidant (mM/l) in testes of rats and after withdrawal period (W.P.). The number of animals was 10 for each group. Values given are Mean ± S.D.

	GSH (µmol/g)		SOD (U/mg protein)		Catalase (U/mg protein)		Total antioxidant (mM/l)	
	Mean ± SD	% Change	Mean ± SD	% Change	Mean ± SD	% Change	Mean ± SD	% Change
Control	7.54±0.60$^{(a)}$		25.37±2.34$^{(a)}$		0.014±0.002$^{(ad)}$		1.22±0.14$^{(a)}$	
Cd	6.70±1.00$^{(bd)}$	*-11.1*	22.79±2.67$^{(bc)}$	*-10.2*	0.021±0.004$^{(bc)}$	*50*	0.95±0.04$^{(bc)}$	*-22.1*
Cd + S	7.50±0.57$^{(a)}$	*-0.5*	23.19±2.14$^{(acd)}$	*-8.6*	0.014±0.003 $^{(a)}$	*0*	1.16±0.21$^{(acd)}$	*-4.9*
Cd + E	7.25±0.72$^{(ad)}$	*-3.8*	21.81±2.83$^{(bd)}$	*-14*	0.013±0.002 $^{(d)}$	*7.1*	0.99±0.10$^{(bd)}$	*-18.9*
E	9.03±0.65 $^{(c)}$	*19.8*	22.72±3.57$^{(bd)}$	*-10.4*	0.014±0.001 (ad)	*0*	1.04±0.10$^{(bd)}$	*-14.8*
W	7.26±0.59$^{(ad)}$	*-3.7*	22.96±2.35$^{(bd)}$	*-9.5*	0.018±0.004 (ac)	*28.6*	1.03±0.19$^{(acd)}$	*-15.6*
F Value	12.79		2		11.11		5.28	
P Value	**< 0.0001**		**0.1**		**< 0.0001**		**< 0.001**	

- One Way Analysis of Variance (ANOVA) was first applied.
- LSD test was used for multiple comparisons, P<0.05 is regarded as significant for homogenous variance equal assumed.
- James-Howell multiple comparison, P<0.05 is regarded as significant for homogenous variance not equal assumed.
- Groups sharing the same letters are not significantly different.

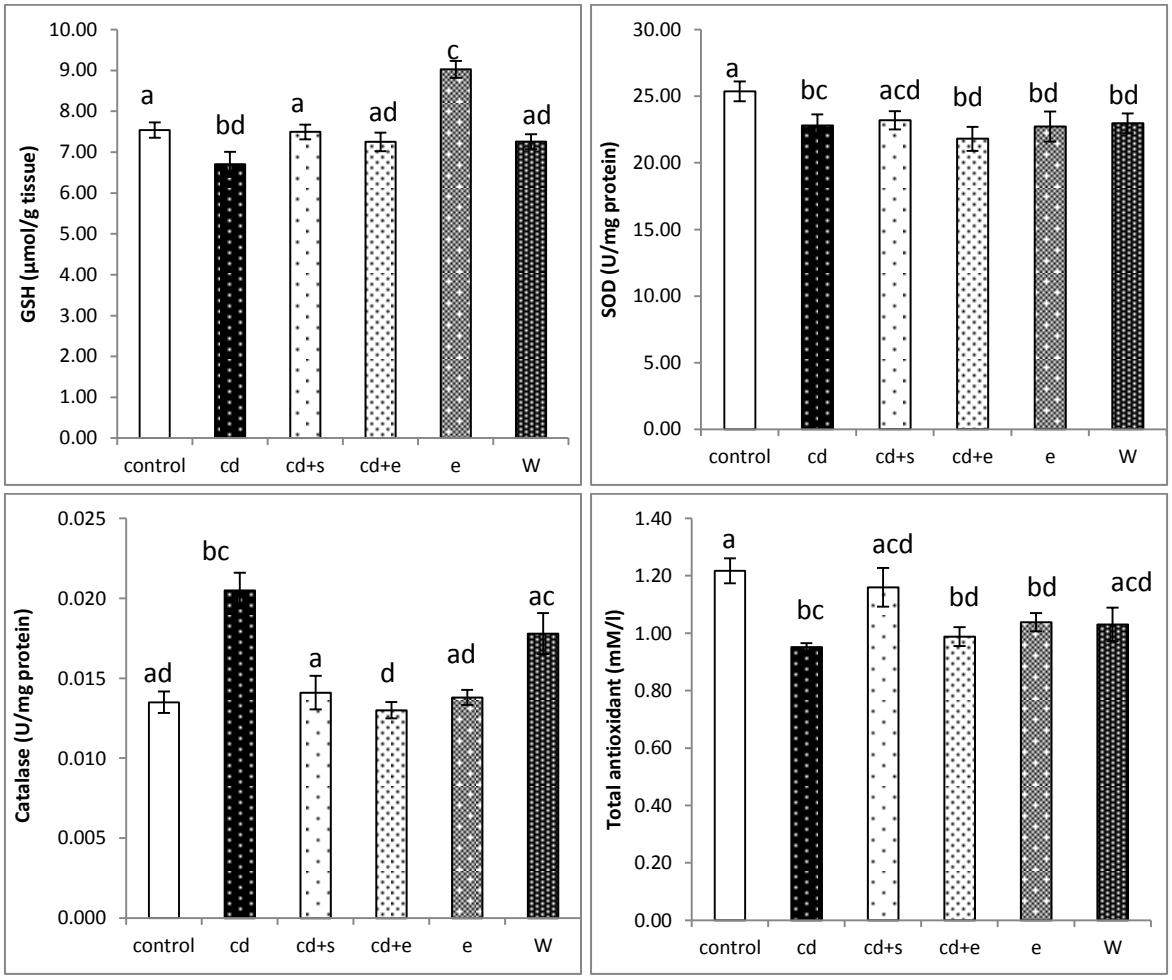


Figure (6) Effect of ($cdcl_2$, vitamin E and stem cells) on antioxidant parameters such as GSH (µmol/g tissue), SOD (U/mg protein), CAT (U/mg protein) and total antioxidant (mM/l) in the testes of rats and after withdrawal period (W.P). Values given are Mean ± S.E. (n=10).

- Groups sharing the same letters are not significantly different.

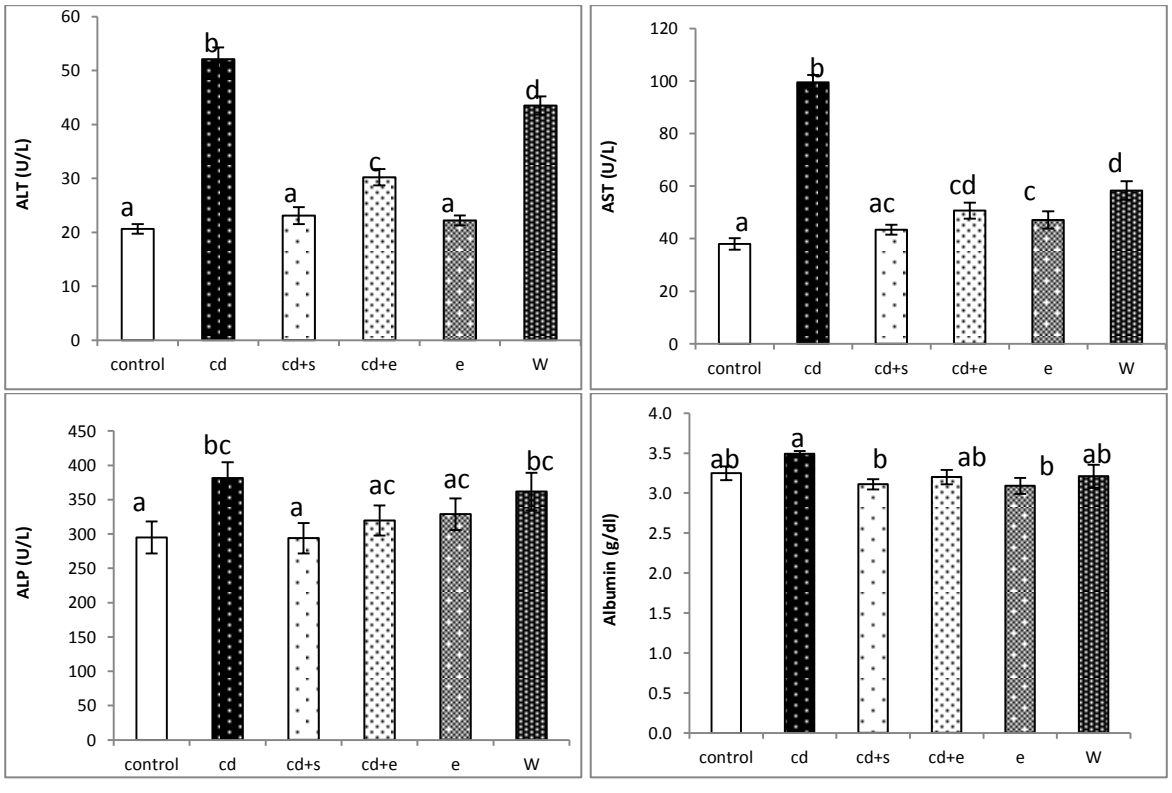
6- Antioxidant parameters

Figure (6) and table (6), illustrates that treatment of rats with $cdcl_2$ for 30 days caused increase of CAT activity to 50.0% of the activity measured in control animals. Stem cells or vitamin E when added to $cdcl_2$ induced a nonsignificant change when compared with control. One-way ANOVA yielded a significant decrease ($P < 0.05$) in the activities of SOD, reduced GSH content and total antioxidant in comparison with control rats (-10.2%, -11.1% and -22.1% respectively). Vitamin E had no significant effect on the cd-induced decrease in the SOD activity (-14.0%) and total antioxidants (-18.9%), While in GSH content had a nonsignificant change when compared with control groups. Induction of stem cells yielded a nonsignificant change in all antioxidant parameters in comparison with control values. After stopping administration of $cdcl_2$, the activity of SOD not retained near their control values (-9.5%).

Table (7): Effect of ($cdcl_2$ 5.0 mg/kg body wt, vitamin E 400 mg/kg body wt and stem cells in a volume of 0.5ml/rat containing 1×10^6 cells) on serum ALT, AST, ALP (U/L), Albumin and T. Protein (g/dl) of rats and after withdrawal period (W.P.). The number of animals was 10 for each group. Values given are Mean ± S.D.

	ALT (U/L)		AST (U/L)		ALP (U/L)		Albumin (g/dl)		T. Protein (g/dl)	
	Mean ± SD	% Change	Mean ± SD	% Change	Mean ± SD	% Change	Mean ± SD	% Change	Mean ± SD	% Change
Control	21.0±2.80(a)		38.0±6.88(a)		294.8±73.47(a)		3.25±0.27(ab)		7.23±0.44(a)	
Cd	52.1±6.9(b)	148	99.5±8.72(b)	161.8	381.2±73.82(bc)	29.3	3.49±0.12(a)	7.4	7.63±0.63(a)	5.5
Cd + S	23.1±5.00(a)	10	43.4±5.95(ac)	14.2	293.8±70.33(a)	-0.3	3.11±0.21(b)	-4.3	7.40±0.61(a)	2.4
Cd + E	30.2±4.83(c)	43.8	50.7±9.63(cd)	33.4	319.4±69.26(ac)	8.3	3.20±0.29(ab)	-1.5	7.53±0.43(a)	4.1
E	22.2±3.00(a)	5.7	47.1±10.49(c)	23.9	328.7±72.37(ac)	11.5	3.09±0.31(b)	-4.9	7.18±0.39(a)	-0.7
W	43.5±5.32(d)	107	58.3±11.20(d)	53.4	361.6±86.76(bc)	22.7	3.21±0.46(ab)	-1.2	7.51±0.88(a)	3.9
F Value	7.18		61.31		2.26		2.35		0.92	
P Value	< 0.0001		< 0.0001		0.06		0.06		0.48	

- One Way Analysis of Variance (ANOVA) was first applied.
- LSD test was used for multiple comparisons, P<0.05 is regarded as significant for homogenous variance equal assumed.
- James-Howell multiple comparison, P<0.05 is regarded as significant for homogenous variance not equal assumed.
- Groups sharing the same letters are not significantly different.



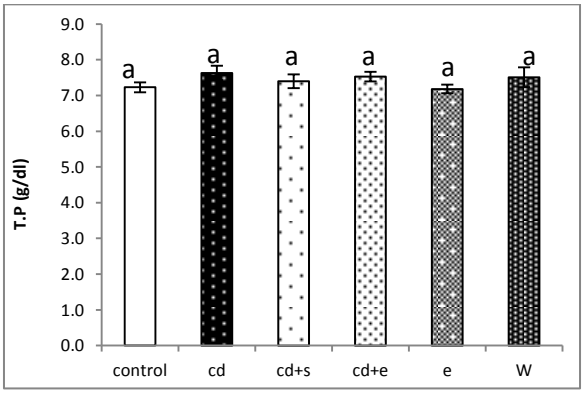


Figure (7) Effect of ($cdcl_2$, vitamin E and stem cells) on serum ALT, AST, ALP (U/L), Albumin and T. Protein (g/dl) of rats and after withdrawal period (W.P). Values given are Mean ± S.E. (n=10).

- Groups sharing the same letters are not significantly different

Table (8): Effect of ($cdcl_2$ 5.0 mg/kg body wt, vitamin E 400 mg/kg body wt and stem cells in a volume of 0.5ml/rat containing 1×10^6 cells) on serum kidney function tests (mg/dl) of rats and after withdrawal period (W.P.). The number of animals was 10 for each group. Values given are Mean ± S.D.

	Creatinin (mg/dl)		Urea (mg/dl)	
	Mean ± SD	*%* Change	**Mean ± SD**	*%* Change
Control	**0.76±0.06**[(a)]		**34.7±3.71**[(a)]	
Cd	**1.04±0.10**[(b)]	*36.8*	**47.4±4.33**[(b)]	*36.6*
Cd + S	**0.76±0.10**[(a)]	*0*	**30.3±4.06**[(c)]	*-12.7*
Cd + E	**0.76±0.10** [(a)]	*0*	**32.9±2.51**[(ac)]	*-5.2*
E	**0.77±0.09**[(a)]	*1.3*	**31.3±2.45**[(ac)]	*-9.8*
W	**0.91±0.06**[(c)]	*19.7*	**40.1±5.13**[(d)]	*15.6*
F Value	**18.14**		**29.12**	
P Value	**< 0.0001**		**< 0.0001**	

- One Way Analysis of Variance (ANOVA) was first applied.
- LSD test was used for multiple comparisons, P<0.05 is regarded as significant for homogenous variance equal assumed.
- James-Howell multiple comparison, P<0.05 is regarded as significant for homogenous variance not equal assumed.
- Groups sharing the same letters are not significantly different.

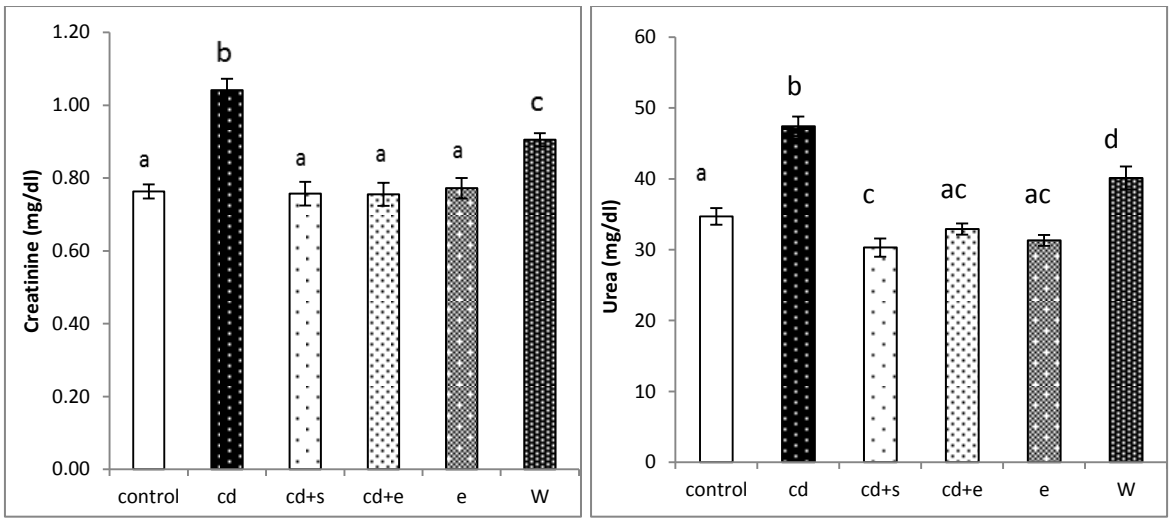


Figure (8) Effect of ($cdcl_2$, vitamin E and stem cells) on serum kidney function tests (mg/dl) of rats and after withdrawal period (W.P). Values given are Mean ± S.E. (n=10).

- Groups sharing the same letters are not significantly different.

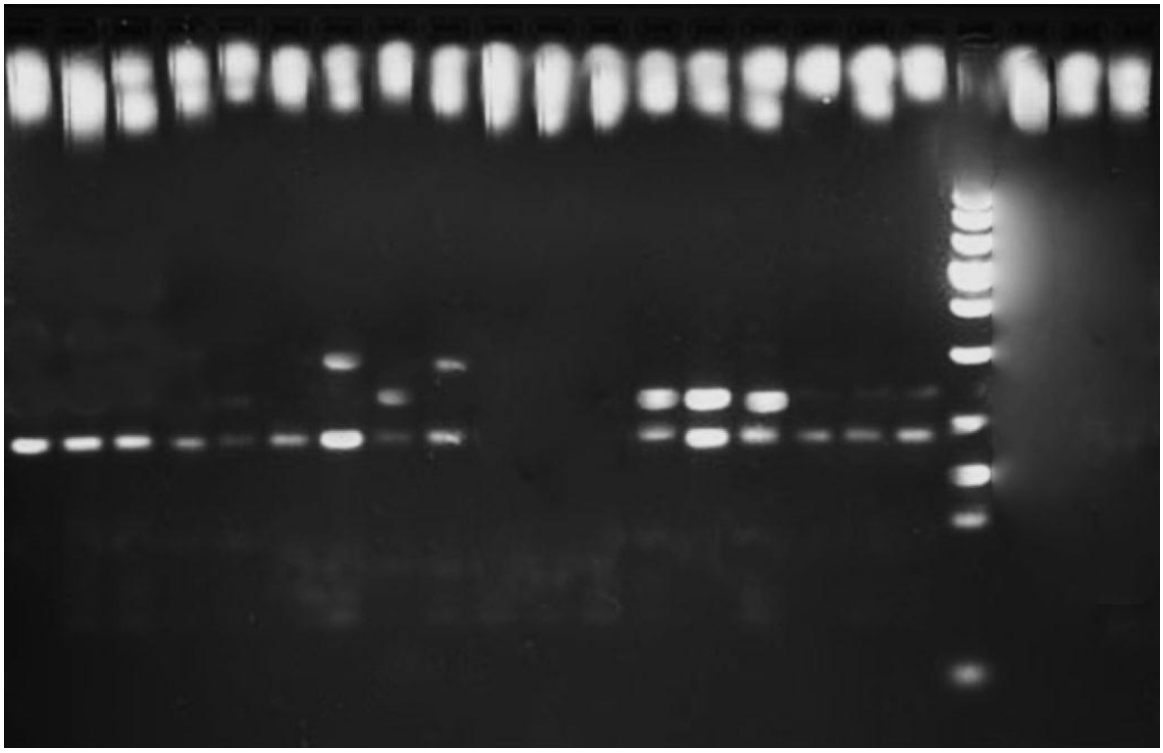


Figure (9) Effect of ($cdcl_2$, vitamin E and stem cells) on DNA fragmentation of rats and after withdrawal period (W.P)

Table (9): Effect of ($cdcl_2$ 5.0 mg/kg body wt, vitamin E 400 mg/kg body wt and stem cells in a volume of 0.5ml/rat containing 1×10^6 cells) on DNA fragmentation of rats and after withdrawal period (W.P.)

Lanes:	Cd		Cd		Cd + S		Cd + S		Cd + E		Cd + S		Cd+E	
Bands	bp	%	bp	%	bp	%	bp	%	bp	%	bp	%	bp	%
1	1054	66.33	1054	47.74	2157	87.99	2157	89.8	2238	85.75	2197	85.64	2183	69.95
2	724	20.61	748	25.57	354	12.01	354	10.15	420	2.052	362	14.35	479	7.498
3	473	13.053	460	26.685					358	12.2			354	22.51
4														
5														
6														
7														
8														
9														
10														
Sum		100		100		100		100		100		100		100
In Lane		100		100		100		100		100		100		100

Cd		Cd		Vit. E		Vit. E		Vit. E		Cd + E	
bp	%	bp	%	bp	%	bp	%	bp	%	bp	%
2183	70.6	2183	69.34	2211	99.97	2183	100	2157	100	2183	63.08
429	17.68	476	13.05							436	22.79
366	11.73	364	17.61							371	14.05
	100		100		100		100		100		100
	100		100		100		100		100		100

W.P		W.P	Cd + S			Cd + S		W.P		LADDER
%	bp	%	bp	%	bp	%	bp	%	bp	%
66.91	2183	70.3	2183	93.14	2183	92.51	2183	76.62	1000	18.21
16.26	431	16.94	368	6.85	368	7.46	431	9.91	900	7.42
16.82	368	12.76					371	13.36	800	7.66
									700	13.28
									600	8.24
									500	9.25
									400	8.56
									300	12.96
									200	6.6
									100	7.53
100		100		100		100		100		100
100		100		100		100		100		100

control		control		control	
bp	%	bp	%	bp	%
2211	100	2211	100	2211	100
	100		100		100
	100		100		100

7- Liver and kidney functions

Figure (7&8) and table (7&8) demonstrates that ALT, AST, ALP, creatinin and urea levels significantly ($P < 0.05$) increased by $cdcl_2$ administration for 30 days, with respect to control rats (148.1%, 161.8%, 29.3%, 36.8% and 36.6% respectively), while total protein, albumin and A/G ratio were not affected. In case of treatment with vitamin E a nonsignificant change occurred in ALP, urea and creatinin, while ALT and AST still elevated (43.8% and 33.4% respectively). On the other hand injection of stem cells decreased the elevation of ALT, AST, ALP and creatinin, while caused a significant decrease in urea when compared with control group (-12.7%). After discontinuation of $cdcl_2$, the levels of ALT, AST, ALP, creatinin and urea not retained near their control values (107.1%, 53.4%, 22.7%, 19.7% and 15.6% respectively).

Discussion

From the present study it could be noticed that induction of infertility by oral administration of $cdcl_2$ was confirmed by a decrease in body weight gain after 30 days. Our results are in accordance with earlier report that showed an association of decreased body weight with $cdcl_2$ of adult rats **(Alvarez *et al.,* 2004 and El-Demerdash *et al.,* 2004).** Our results revealed that, treated rats with $cdcl_2$ (5 mg/kg body wt/day) for 30 days caused reductions in the weight of genital sex organs (right and left testis, epididimus, prostate gland and seminal vesicles) and this may be due to the oxidative damage induced by cadmium chloride. The oxidative damage might have destroyed most of the germ cells either due to the membrane damage or macromolecular degradation incurred by ROS leading to fall in testicular weight **(Agarwal *et. al.,* 1997).**

The decrease in testes and epididymis weights may be due to the generation of free radicals, resulting in oxidative deterioration of proteins, lipids and DNA induced by $cdcl_2$ and this caused atrophy to testes **(Shen & Sangiah, 1995; Hussain *et al.,* 1997)**. **(WHO, 1992; Shen & Sangiah, 1995; Wang *et al.,* 2007)** illustrate that $cdcl_2$ been to induce necrotic degeneration of testicular tissues, which may contribute to weight loss of testis. Moreover, the weight of the testis is largely dependent on the mass of undifferentiated spermatogenic cells. Thus, the observed weight loss of the testis may be due, in part, to the adverse effect of $cdcl_2$ on the number of germ cells and elongated spermatids **(Yang *et al.,* 2006)**. Exposure of rats to $cdcl_2$ resulted in a significant reduction in seminal vesicle **(Amara *et al.,* 2008; Monsefi *et al.,* 2010 and Alaee *et al.,* 2014**).

From the present study it could be noticed that vitamin E (α-tocopherol) protects critical cellular structures against damage caused by oxygen-free radicals

and reactive products of lipid peroxidation. It has been reported that lipid peroxidation was prevented by vitamin E **(Meydani, 1995)**. Also, vitamin E is considered a major free radical chain-breaking antioxidant and can interfere with the initiation and progression of Cd-induced oxidative damage. Vitamin E is the primary liposoluble antioxidant, which may have an important role in scavenging free oxygen radicals and in stabilizing the cell membranes, thus maintaining its permeability **(Beyer, 1994; Chen H & Tappel, 1995 and Ibrahim *et al.,* 2000)**.

Most human mesenchymal somatic cells (hMSCs) are present in the bone marrow, small stores of stem cells are distributed throughout various tissues as reserves for wound repair and scar formation **(Horwitz *et al.,* 1999)**. When tissue damage occurs, specific endocrine signals are released from the injury site that mobilizes hMSCs from bone marrow to the location of damage **(Fox *et al.,* 2007)**. Furthermore, small amounts of hMSCs are retained in the blood circulation and have the ability to home to injury sites for tissue repair **(Prockop, 1997 and Studeny *et al.,* 2004)**.

In the present study, serum levels of LH and testosterone were reduced in infertile rats and elevation of FSH and prolactin. The above observation was running parallel with finding of **Gunnarsson *et al.* (2003)** who reported that Cd caused a decrease in testosterone production through the reduction of testicular luteinizing hormone (LH) receptor, messenger ribonucleic acid (mRNA) and cyclic adenosine monophosphate (cAMP) level in rats. They reported that Cd exposure can interfere with the initial steps of hormone-mediated regulation of the testis which is important for normal gamete production. Moreover, **Norman Barwin, (1978)** found that any chemical agent suppressing the secretion of pituitary gonadotropins will produce antispermatogenic and antifertility effects; impaired secretion of luteinizing hormone by the pituitary results in deficient androgen secretion by the testes, cessation of spermatogenesis and loss of libido. Steroidal hormones may inhibit the secretion of pituitary gonadotropins via negative feedback to the hypothalamus, which results in inhibition of spermatogenesis and testosterone secretion. Serum LH levels are markedly elevated in 2/2 animals, which could be due to a loss of estradiol (female) and testosterone (male) negative feedback and/or loss of negative LH feedback on its own secretion through decreased hypothalamic GnRH levels in both sexes **(Lei & Rao, 1994; Mores *et al.,* 1996 and Lei & Rao, 1997)**.

Our result show increased in plasma levels of FSH after cadmium administration for 30 days. This increase agreed with **Hassanin & Safwat (2014).** This finding could be due to the accumulation of cadmium in the testis. It was shown that cadmium affects Sertoli cell activity by decreasing inhibin synthesis and release. Thus, the increased plasma levels of FSH could be explained, as this peptide is the main inhibitory signal for FSH secretion **(De souza Predes *et al.,* 2010)**. Our observations were in line with changes observed by **(Saeed, 2013). Antonio *et al.* (1999)** Authors described a marked reduction of seminiferous tubular diameter after the higher dose of cadmium, along with the conspicuous decrease of the tubular volume density, which means that cadmium caused a significant reduction in the relative seminiferous tubule length **(Selgas *et al.,* 1997)**. These data confirm that the severity of cadmium-induced damage at the testicular tissue and the seminal vesicles **(John *et al.,* 1984**), which lead to decrease in reproductive organ weight and cause changes in the diameter of seminal vesicle and interstial space **(Coni *et al.,* 1992).**

The results of the present study also declare that induction of infertility by $cdcl_2$ significantly increased in lipid peroxidation rate and fructose in semen and decreased sperm counts and their motility which agree well with the results of previous studies **(Hsu *et al.,* 1998; Oteiza *et al.,* 1999 and El-Demerdash *et al.,* 2004)** whose reported that cadmium replaces calcium in calcium-binding proteins, causing disruption or cessation of activity, which can lead to oxidative stress, perhaps due to the low production of sperm in testes and that could be related to the low levels of gonadotrophins and/or plasma testosterone level **(Steinberger, 1971 and Steinberger, 1975)**. Metal ions such as transition metals cause cellular damage via formation of highly reactive oxygen free radicals $^{\cdot}OH$, which is derived from O_2^-, and hydrogen peroxide (H_2O_2), by the Haber–Weiss reaction **(Halliwel & Gutteridge, 1984)**. Also **Aitken *et al.* (1993a)** reported that human spermatozoa are known to be susceptible to loss of motility in the presence of exogenous H_2O_2, as a consequence of lipid peroxidation.

This susceptibility of human spermatozoa to oxidative stress is a consequence of the abundance of unsaturated fatty acids in the sperm plasma membrane, the presence of which gives this structure the fluidity it needs to engage in the membrane fusion events associated with fertilization. Unfortunately, the presence of double bonds in these molecules makes them vulnerable to free radical attack and the initiation of a lipid peroxidation cascade. Studies concerning the chemistry of lipid peroxidation in human spermatozoa **(Aitken et al., 1992b)** imply that once this process has been initiated, its propagation is impeded, leading to the accumulation of lipid peroxides in the sperm plasma membrane. The extent to which lipid peroxidation occurs will depend upon the antioxidant strategies available to the spermatozoon. The loss of sperm function that results from lipid peroxidation reflects the negative impact that lipid peroxides have on membrane fluidity and the activity of key membrane-bound enzymes, such as Ca^{2+}/Mg^{2+}-ATPases, which are involved in maintaining calcium homeostasis within these cells **(Irvin, 1996)**. Spermatozoa have been considered to be highly susceptible to lipid peroxidation in the presence of elevated reactive oxygen species (ROS) level, due to the abundance of polyunsaturated fatty acid (PUSFA) in their membranes **(Peltola *et al.,* 1992)**. Increase ROS

formation due to lipid peroxidation and a compromised antioxidant defense system may be caused mid-piece abnormalities and decreased sperm count and motility **(El-Taieb *et al.,* 2009 and Shiva *et al.,* 2011)**. The process of fructose formation in seminal vesicle is initiated and controlled by testicular androgens **(Brooks, 1979)**. Hormonal deficiency causes a decrease or even disappearance of seminal fructose and a compensatory treatment with androgens restores the ability of the accessory glands to produce this sugar **(Hamdan noor *et al.,* 1998)**. Increased production of reactive oxygen species (ROS) and inflammatory cytokines seems to be crucial in the pathogenesis of tissue injury in response to cadmium exposure (**Kayama *et al.,* 1995 and Acharya *et al.,* 2008**).

The antioxidant such as Vitamin E may act synergically, prevent lipid peroxidation and cell destruction **(Kim & Combs, 1993; Beyer, 1994; Chen & Tappel, 1995; Griffith, 1999; Sies, 1999; Ognjanovi ´ c *et al.,* 2003 and Acharya *et al.,* 2008). El-Far *et al.* (2012)** reported that 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. In the present study, MSC group associated with a significantly higher sperm count and motility along with a concomitant increase in testosterone level, compared to the $CdCl_2$ treated rats group. Hence, it could be concluded that MSCs might have a potential role in treat male infertility and testosterone deficiency. It could also be proposed that the beneficial effects of MSCs may be due to differentiate into male germ cells as reported by **Nayernia *et al.* (2006)**.

The induction of oxidative stress in infertile rats in the present study is reflected by increased production of TBARS, a measure of lipid peroxidation in the homogenates of testis. **Kappus, 1985 and Janero, (1990)** reported that cd-induced lipid peroxidation implies elevated lipid peroxides in the murine testes and is an indicator of local oxidative stress. Oxygen radicals characteristically react with polyunsaturated fatty acid residues in membranes leading to an array of lipid peroxidation products, many of which also damage proteins and DNA. The higher membrane lipid content of Leydig cell mitochondria and microsomes may explain the susceptibility of the testis to lipid peroxidation in Cd-exposed mice **(Dobrestov *et al.,* 1977; Georgiou *et al.,* 1987 and Hall, 1995)**. **Eneman *et al.* (2000)** illustrated that lipid peroxidation is considered the primary mechanism for cd-toxicity despite its inability to directly generate free radicals under physiological condition **(Eneman *et al.,* 2000)**. The displacement of iron and copper from various intracellular sites by cadmium increases the concentration of ionic iron and copper which intern cause oxidative stress through Fenton reaction producing hydroxyl radical species which are believed to initiate lipid peroxidation **(Yiin *et al.,* 1999)**. Decomposition products of lipid hydroperoxide such as malondialdehyde and 4-hydroxy nonenal can cause chaotic cross-linkage with proteins and nucleic acids which plays an important role in the process of carcinogenesis.

In view of the fact that cadmium is a non-redox metal, it always adopts a single oxidation state and is not a strong inducer of reactive oxygen species. The major mechanism behind the case is the disruption of electron transfer chain and the induction of mitochondrial (ROS) **(Wang *et al.,* 2004)**. Therefore, some authors have postulated that antioxidants should be one of the important components of an effective treatment of cadmium poisoning **(Shaikh *et al.,* 1999; Casalino *et al.,* 2002 and El-Demerdash *et al.,* 2004).** A synergistic effect of vitamin E in the protection of biomembranes from oxidative attack has been widely discussed. Vitamin E is known to readily reduce alkyl peroxy radicals of unsaturated lipids **(Burton *et al.,* 1983).** Supplementation of α-tocopherol increases the availability of the vitamin to ameliorate oxidative stress. This intervention could restore the activity of antioxidant enzymes to enable normal germ cell differentiation programs and protect sperm count profile and morphologies during Cd exposure **(Acharya *et al.,* 2008)**.

In the present study, a high lipid peroxidation with a concomitant increase in CAT enzyme and decrease in the enzymatic antioxidant status, reduced GSH, SOD and total antioxidant were recorded in testicular tissue of infertile rats. These enzymes are closely related to direct elimination of ROS **(Sahoo *et al.,* 2008)**. These results of the present study was in accordance with previous study that cadmium depletes glutathione and protein bound sulphadryl groups in the testes, resulting in enhanced production of ROS such as superoxide ion, hydroxyl radicals and hydrogen peroxide **(Stohs *et al.,* 2000)**. In fact, it has been reported that the sulfhydryl group of cysteine moiety of glutathione has a high affinity for metals such as $cdcl_2$, forming thermo-dynamically stable mercaptides complexes which are inert and excreted via the bile **(Wang & Ballatori, 1998; Mohanpuria *et al.,* 2007)**. $Cdcl_2$ may cause an increase in oxidative stress by binding to sulfhydryl groups of proteins and by depleting glutathione resulting in increase of lipid peroxidation **(Figueiredo-Pereira *et al.,* 1998; Stohs *et al.,* 2001; Watjen & Beyersmann, 2004 and Siu *et al.,* 2009)**. Superoxide dismutase (SOD) and catalase (CAT) are the two major enzymes that play an important role in eliminating reactive oxygen species formed during bioactivation of xenobiotic in hepatic tissues **(Michlin & Bendich, 1987)**. SOD scavenges the superoxide radicals whereas CAT catalyzes the breakage of toxic H_2O_2 produced in the cell to O_2 and H_2O. The SOD and CAT are easily inactivated by lipid peroxide or reactive oxygen species **(Halliwell & Gutteridge, 1987).**

Quig D (1998) observed that the induction of testicular oxidative stress is due to excess accumulation

of intracellular ROS, the high levels of protein carbonyl content and lipid peroxidation in the testes. $Cdcl_2$ is a divalent heavy metal having strong affinity towards the thiol groups. During $cdcl_2$ exposure most of the antioxidant enzymes become inactive due to its binding to the active sites of the enzyme containing–SH groups. **Casalino *et al.* (2000)** showed that most of the antioxidant enzymes become inactive due to the displacement of the metal co-factors from the active sites.

Our results showed that the activity of SOD was lower in the testes of Cd-exposed rats. The interaction between Cd and essential trace elements could be one of the reasons for decreased antioxidant enzymes in the rat testis **(Oteiza *et al.,* 1999)**. In physiological conditions, SOD is an important defense enzyme, which converts $O_2^{\cdot-}$ to H_2O_2 and thus protects against superoxide induced damage **(Kono & Fridovic, 1982 and Mates, 2000)**. The toxic effect of Cd on SOD activity could result from interactions between Cd and Zn, Cu or Mn in the SOD molecule. The basis of Cd toxicity is its negative influence on enzymatic systems of cells, resulting from its substitution for divalent mineral elements (Zn^{2+}, Cu^{2+} and Mn^{2+}) in metalloenzymes and its very strong affinity to biological structures containing –SH groups, such as proteins, enzymes and nucleic acids **(Shukla *et al.,* 1987; Patra *et al.,* 1999 and Acharya *et al.,* 2008).**

Vitamin E (α-tocopherol) inhibits peroxidation of membrane lipids by scavenging lipid peroxyl radicals, as a consequence of which it is converted into α-tocopheroxyl radical. This radical is thought to be either recycled to α-tocopherol by interacting with soluble antioxidants, such as ascorbic acid, or irreversibly oxidized to α-tocopherylquinone. In fact, α-tocopherylquinone may act as a potent anticoagulant and as an antioxidant through its reduction to hydroquinone **(Arita *et al.,* 1998).** However, administration of MSC/BM extract significantly prevented the influence of $cdcl_2$ on antioxidative system. It decreased MDA and SOD and concomitantly increased the activities of CAT level in testes tissue. These results clearly demonstrate the antiperoxidative role of the MSC/BM. The in vivo protection by MSC/BM against $cdcl_2$ induced oxidative damage may be because of its free radical scavenging potential. The specific responses of mesenchymal stem cells to oxidative stress may play a crucial role in regulation of tissue homeostasis as well as regeneration of organs after oxidative injury **(Burova *et al.,* 2013 and Hassan & Alam, 2014)**. It could also be because of direct scavenging/neutralization of the free radical or induction of the endogenous antioxidant enzymes such as CAT and SOD.

The data obtained from the present study show that infertility caused increase in DNA fragmentation after administration of $cdcl_2$ for 30 days. And this compatible with several investigators who recorded an increase in DNA fragmentation in adult rats **(Monsefi *et al.,* 2010)** who reported that cadmium is able to inhibit sperm chromatin condensation. Immature sperm cells, sperm DNA fragmentation occurs as a result of abnormal chromatin condensation. The tight packaging of chromatin, based on condensation and substitution of histones with protamines, is extremely important as mature sperm is unable to repair DNA damage **(Laberge & Boissonneault, 2005 and De Ambrogi *et al.,* 2006)**. Free radicals have the ability to directly damage sperm DNA by attacking the purine and pyrimidine bases and the deoxyribose backbone. Normally, sperm DNA is tightly packaged by protamines protecting it from free radical attack. However, infertile men often exhibit deficient protamination, leaving the sperm DNA particularly vulnerable to ROS attack **(Oliva, 2006)**. Alternatively, free radicals can initiate apoptosis within the sperm, leading to caspase-mediated enzymatic degradation of the DNA **(Kemal Duru *et al.,* 2000; Wang *et al.,* 2003; Moustafa *et al.,* 2004 and Villegas *et al.,* 2005).** ROS may cause varying degrees of sperm dysfunction, depending on the extent of oxidative stress. Damage from ROS occurs primarily through two routes: First, ROS may be responsible for the DNA fragmentation commonly seen in the spermatozoa of infertile men by causing single- and double-stranded DNA breaks **(Kodama *et al.,* 1997)**. Second, higher levels of ROS also may cause damage through a chain of chemical reactions that result in lipid peroxidation of the sperm plasma membrane **(Alvarez *et al.,* 1987)**.

Vitamin E (α-tocopherol) is naturally occurring antioxidants that play important roles in animal health by inactivating harmful free radicals produced through normal cellular activity and from various stressors. The antioxidant function of this micronutrient could, at least in part, enhance immunity by maintaining the functional and structural integrity of important immune cells **(Chew, 1995; Pregiosi *et al.,* 1998; Yousef *et al.,* 2003 and El-Demerdash *et al.,* 2004).** One theory of tissue repair holds that organ injury is "sensed" by stem cells that migrate to the site of damage and differentiate into organ-specific cells, promoting structural and functional repair **(El Shafai *et al.,* 2011 and EL-Attar *et al.,* 2012)**. **Cakici *et al.* (2013**) reported that The MSC cells were found both outside of the basal compartment and in the seminiferous tubules, supporting the idea that MSCs might have functioned in reestablishment of spermatogenesis by two ways: MSCs' differentiation into sperm, or maintenance of the spermatogonial stem cells. These results showed the MSCs could be both rich and functional source for the infertility treatment.

The increase in serum AST and ALT activities are in agreement with the findings of **Rana *et al.* (1996)** who found that cadmium caused alterations in liver transaminases of rats. The increase in serum AST and ALT activities indicated an active transamination of amino acids and operation of keto acids which are probably fed into tricarboxylic acid cycle (TCA) for oxidation **(Knox & Greengard, 1965)**. Therefore, the increase in the activities

of AST and ALT in plasma is mainly due to the leakage of these enzymes from the liver cytosol into the blood stream **(Navarro *et al.,* 1993)**, which gives an indication of the hepatotoxic effect of $cdcl_2$. Their estimation in the serum is a useful quantitative marker of the extent and type of hepatocellular damage **(Mitra *et al.,* 1998).** The increased blood urea and creatinine are in agreement with the results obtained by **Rana *et al.* (1996)** in male rats. Elevated blood urea is known to be correlated with an increased protein catabolism in mammals and/or the conversion of ammonia to urea as a result of increased synthesis of arginase enzyme involved in urea production **(Harper *et al.,* 1979).**

In conclusion, this study indicates that stem cells play an important role in recovering of testicular function of adult rats. Supporting the idea that MSCs might have functioned in reestablishment of spermatogenesis by differentiation into sperm. MSCs have tremendous potential for regenerative medicine; bone marrow-derived mesenchymal stem cells are capable of differentiating into germ cells and Leydig cells in the testis. In the present study, MSCs modulated the decline of serum testosterone level induced by $cdcl_2$ and reached approach within control values. Because Leydig cells are responsible for testosterone production, stem cell transplantations may replace the need of life-long testosterone supplementation in male hypogonadism.

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