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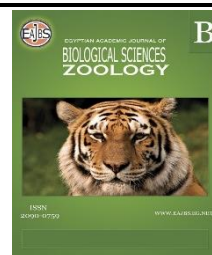


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## Cadmium-Induced Testicular Damage in Rats: Modulatory Role of Stemenhance® Against Oxidative Stress

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One of the main environmental toxins that pose a risk to human health is cadmium (Cd) which especially affected human fertility. This investigation aimed to study whether the Stem Enhancer® was able to treat cadmium chloride-evoked testicular toxicity or not. About 36 male rats were divided into 6 groups equally: the control group, tween80 group, and StemEnhance® group received 198mg/kg b. w. /days orally, cadmium chloride group (CdCl<sub>2</sub>) received 15 mg/kg b.w. / day orally for 45 consecutive days, CdCl<sub>2</sub>+distilled water group received CdCl<sub>2</sub> 15mg/kg/day orally for 45 days, and then it received distilled water orally for another 45 days and CdCl<sub>2</sub>+StemEnhance®group received CdCl<sub>2</sub> 15mg/kg/day orally for 45 days, and then it received StemEnhance® 198 mg/kg body weight /days orally for another 45 days. The results demonstrated that CdCl<sub>2</sub> reduced sperm motility, count, and relative weight of testes and increased the percentage of dead sperm and sperm abnormalities and caused histopathological alterations. CdCl<sub>2</sub> and decreased level of testosterone, FSH and testicular total antioxidant level in serum. However, the sperm profile of rats in the StemEnhance® treated group was improved, as the testosterone, FSH and total antioxidant capacity increased. So by inhibiting oxidative stress and acting as a bone marrow stem cell mobilizer, StemEnhance®, according to our findings, may be able to treat testicular damage caused by cadmium chloride.

### INTRODUCTION

Infertility is the inability of couples to have babies after a year of regular unprotected intercourse, this affects about 10 - 15% of human couples. WHO statistics recorded that male factors are responsible for 20–30% of infertility cases (Babakhanzadeh *et al.*,2020) and it is considered a great problem and different treatments are investigated (Un *et al.*, 2020). One of the causes of infertility is exposure to heavy metals which have a deteriorative effect on sperm and fertility in humans or animals (Ghafouri *et al.*, 2021).

Heavy metal (cadmium) has an impact on numerous systems in both humans and animals. Exposure to cadmium occurs during ingestion of water, food contaminated with cadmium, also, through normal inhalation and smoking and it has a long biological half-life reach 20 years (Sarkar *et al.*, 2013). Cadmium in the human is a causative agent of many

dangerous health problems, including morphological changes in the reproductive organs including seminal tubules necrosis and interstitial edema which cause decreasing in testosterone synthesis and hampered spermatogenesis (Skolarczyk *et al.*, 2018). Mechanisms by which Cd-induced organ toxicity are inflammation, oxidative stress, chromosomal aberration and apoptotic cell death (Sarkar *et al.*, 2013 and Kaur *et al.*, 2020). Because of the significant role that oxidative stress plays in cadmium toxicity, various organs including kidneys, liver, lungs, bones, and reproductive organs may suffer from physiological damage (Rani *et al.*, 2013). Oxidative stress occurred due to an imbalance between oxidation and antioxidant levels as the antioxidant consumption increased and reactive oxygen species (ROS) accumulated. Hydrogen peroxides, superoxide anions, and hydroxyl radicals damage DNA, proteins, and lipids and cause oxidative stress by depleting GSH levels and impeding the antioxidant defense system created by cadmium toxicity (Fang *et al.*, 2021).

StemEnhance<sup>®</sup> is an extract that comes from the blue-green alga *Aphanizomenon flos-aquae* and is used as a health supplement (Dirikolu *et al.*, 2010). Consumption of StemEnhance<sup>®</sup>, the herbal extract from *Aphanizomenon flos-aquae* (AFA) can increase the part of stem cells in the circulation as L-selectin ligand (LSL) isolated from AFA responsible for increasing the circulating bone marrow stem cells (Jensen *et al.*, 2007). Migratose is another StemEnhance<sup>®</sup> extract present in a polysaccharide-rich fraction and supports the stem cells' migration from the blood into tissues (Dirikolu *et al.*, 2010) so StemEnhance<sup>®</sup> may cause mobilization of stem cells from the bone marrow (Drapeau *et al.*, 2010, El-Akabawy and El-Mehi 2015). This stem cell develops into various cell types in the body with special functions. In addition to differentiating into different types of cells, they also produce amounts of cytokines and growth factors which may mediate endogenous regeneration through activation of other stem cells as well as promote neovascularization, anti-inflammation and anti-apoptosis (Abdelaziz *et al.*, 2019 and Wen *et al.*, 2011) and exert antioxidant response (Ayatollahi *et al.*, 2014). In relation to our study, MSCs (bone marrow mesenchymal stem cells) can repair testicular damage and ameliorate both histopathological and biochemical abnormalities. MSCs can improve gonadotoxicity by different mechanisms such as anti-inflammatory, antioxidative, and antiapoptotic (Sherif *et al.* 2018 and Elbaghdady *et al.*, 2018).

## MATERIALS AND METHODS

### 1-Animal:

We used Sprague-Dawley rats (36 adult males, weighing  $200 \pm 20$  g). They get from the breeding colony and then maintained at the animal house of the Egyptian Drug Authority (EDA, Giza, Egypt), Formerly NODCAR. Animals received a standard diet and water was allowed *ad libitum*. Adaptation period for 2 weeks subjected to animals in the animal house before starting the experiment. The experiment was maintained at a temperature range from 21 to 24°C and 40–60% humidity with 12 hrs light-dark cycle. Experimental procedures were conducted in accordance with the ethical guidelines for investigations in laboratory animals and were approved by the standard guidelines of EDA (Approval number: NODCAR/I/30/2022) in handling the experimental animals and conforms to the Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

### 2. Chemicals and Drugs:

**2.1.** Cadmium chloride was obtained from LOBA chemie dissolved in dist. H<sub>2</sub>O and given daily with a dose of (15 mg/kg/day; Orally) according to a previous study (Farombi *et al.* 2012).

**2.2.** StemEnhance<sup>®</sup> distributed by Stem Tech Health Science, Inc Pembroke Pines, FL 33028 was dissolved in 1% tween<sup>80</sup> with a dose of 198mg/kgb. wt equivalent to a human dose of

2200 mg/day after dose conversion according to the table of (Paget and Barnes 1964).

### 3. Experimental Design:

Thirty-six rats were divided randomly into six groups (each with 6 animals).

**Group I:** normal control and were given only distilled water.

**Group II:** vehicle for stem enhancement and received 1% Tween<sup>80</sup> as a vehicle to StemEnhance<sup>®</sup>.

**Group III:** drug-alone treated group and was treated with StemEnhance<sup>®</sup> in a dose of 198mg/kg/day orally.

**Group IV:** treated with cadmium chloride in a dose of 15mg/kg/day orally. All these groups received treatment for 45 days.

**Group V:** treated with cadmium chloride in a dose of 15mg/kg/day orally for 45 days then, received distilled water for 45 days.

**Group VI:** treated with cadmium chloride in a dose of 15mg/kg/day orally for 45 days then received StemEnhance<sup>®</sup> in a dose of 198mg/kg/day orally for 45 days.

### Tissue and Blood Sampling:

At the end of the experiment, Rats were weighed, given mild anaesthesia and collect blood samples were in order to estimate the serum testosterone and FSH concentrations. Thereafter, animals euthanized by decapitation, testes and epididymis were quickly extracted, rinsed with ice-cold saline and cleaned from the adhering tissue. The right side testis decapsulated and split into two pieces (kept at -80 °C) for assessment of total antioxidant capacity. The left testis was split into two parts one part was preserved in 10% formalin for histopathological evaluation and the other part was cut into small pieces and immediately fixed in 2.5% 1M phosphate-buffered glutaraldehyde at 4°C for 2 hours for electron microscope study.

#### 1. Determination of Serum Testosterone:

The serum was separated by centrifugation and then stored at -80 °C. The testosterone level in the serum was measured using a (MyBioSource<sup>®</sup> USA) testosterone ELISA kit according to the manufacturer's protocol.

#### 2. Determination of Serum FSH:

The FSH (follicle stimulating hormone) level in the serum was measured using a (MyBioSource<sup>®</sup> USA) FSH ELISA kit according to their manufacturer's protocol.

#### 3. Determination of Total Antioxidant Capacity:

Testes homogenate were used for the estimation of total antioxidant capacity using Bio-diagnostic Co., (Cairo, Egypt) kits (koracevic *et al.*, 2001).

#### 4. Percentage of Sperm Count, Motility, Vitality and Abnormalities:

Epididymal content was obtained immediately after sacrificing rats, then the percentage of sperm count, motility, vitality and abnormalities were estimated according to the method described by (Bearden and Fluquary 1980).

#### 5. Histopathological Examination:

Testes that were taken and fixed in 10% formalin solution are used. Washing was done in tap water then serial passages with graded ethyl alcohol for dehydration. Paraffin tissue blocks sectioned at 4 microns of thickness by microtome. The obtained tissue sections were stained by Haematoxylin and Eosin stain according to the method described by **Banchroft *et al.* at 1996** to examine it by using the light microscope.

#### 6. Electron Microscopic Examination:

For TEM examination (transmission electron microscope), small pieces of the testis (about 1mm) and immediately fixed in 2.5% 1M phosphate-buffered glutaraldehyde at 4°C for 2 hours, rinsed in 0.1M phosphate-buffered then post-fixed in phosphate-buffered 1% osmium tetroxide for 1 hour at 4°C. After that, it dehydrated in ascending grades of ethanol. After immersion in propylene oxide, the specimens were embedded in an epoxy resin mixture (Gupta, 1983). Semi-thin section (1microne thick) cut then stained with toluidine

blue, then examined with the light microscope. After the proper areas were selected, ultrathin sections (60-90nm) were cut and picked up on copper grids; the section was double stained with uranyl acetate and lead citrate (Reynolds,1963). And finally, the stained sections were examined with a JEOL 1010 transmission electron microscope at the Regional Center for Mycology and Biotechnology (RCMB), AL-Azhar University.

#### **7. Statistical Analysis:**

Results have been analyzed by the prism program version (5). Comparison between more than two different groups was carried out using a one-way ANOVA analysis of variance followed by Tukey-Kramer's Multiple Comparison Test according to Armitage and Berry in 1987 where  $P < 0.05$  significant. All the values were measured as means  $\pm$  standard errors of the means (S.E.M).

## **RESULTS**

### **Effect of StemEnhance<sup>®</sup> on the Relative Weight of Testes, Sperm Motility, Count, Percent of Dead Sperm and Abnormalities in Cadmium Chloride Treated Group:**

Cadmium administration induced a considerable reduction in the relative weight of testes, sperm motility and sperm count by 37%, 67% and 53% respectively and caused a significant increase in the percentage of dead sperm and sperm abnormalities by 102% and nearly 4 folds respectively as compared to normal control. However, Stemenhance<sup>®</sup> administration for 45 days after cadmium ( $\text{CdCl}_2 + \text{Stemenhance}^{\text{®}}$ ) attenuated the deleterious effect of cadmium chloride by increasing the relative weight of testes, sperm motility and sperm count by 51%, 198% and 92% respectively and produced a valuable decrease in the percentage of dead sperm and abnormal sperm percent by 46% and 73% respectively as compared to cadmium group. In contrast, distilled water administration for 45 days after cadmium ( $\text{CdCl}_2 + \text{distilled water}$ ) caused no significant difference in the relative weight of testes, sperm motility, sperm count, percentage of dead sperm and sperm abnormalities as compared to cadmium group.  $\text{CdCl}_2 + \text{StemEnhance}^{\text{®}}$  group generated a significant increase in (relative weight of testes, sperm motility and sperm count) by 43%, 170% and 90% respectively and caused a significant decline in the percentage of dead sperm and sperm abnormalities by 43% and 73% respectively versus to ( $\text{CdCl}_2 + \text{distilled water}$ ). Treatment with StemEnhance<sup>®</sup> alone not produced any significant alterations in the relative weight of testes, sperm motility, sperm count, percentage of dead sperm and sperm abnormalities when compared with the control group (Figs.1&2).

### **Effect of StemEnhance<sup>®</sup> on Testosterone and FSH in Cadmium Chloride Treated Group:**

In cadmium treated group, there is a significant decrease in testosterone and FSH in serum by 58% and 37% occurred compared to the control group. Treatment with StemEnhance<sup>®</sup> amended the alterations caused by cadmium by increasing the serum testosterone and FSH by 116% and 50% compared to the cadmium group, in spite of that, no significant difference between  $\text{CdCl}_2 + \text{distilled water}$  group and the cadmium group. StemEnhance<sup>®</sup> administration for 45 days after cadmium-induced a significant difference in the serum testosterone and FSH by 45% and 46% compared to ( $\text{CdCl}_2 + \text{distilled water}$ ). StemEnhance<sup>®</sup> group has no significant difference in the serum testosterone and FSH levels compared to the control group (Fig. 3).

### **4. Effect of Stemenhance<sup>®</sup> on Total Antioxidant Capacity in Cadmium Chloride Treated Group:**

Cadmium caused a significant decrease in total antioxidant capacity by 44% when compared with the control group. total antioxidant capacity in testes of animals that received ( $\text{CdCl}_2 + \text{StemEnhance}^{\text{®}}$ ) increased 104% compared to those in the cadmium group but ( $\text{CdCl}_2 + \text{distilled water}$ ) not corrected the cadmium effect. StemEnhance<sup>®</sup> administration for

45 days after cadmium can alleviate this effect by increasing total antioxidant capacity by 78% compared to the (CdCl<sub>2</sub>+ distilled water) group. Finally, StemEnhance® alone increased total antioxidant capacity by 31% compared to the control group (Fig.4).

#### **5-Histopathological Finding:**

To confirm our biochemical and sperm profile finding we applied histopathological examination, we found that the testis of rats in the normal control, tween<sup>80</sup> and StemEnhance® groups appeared with normal seminiferous tubules, spermatogenic cells and interstitial cells (Fig. 5 a, b and c). Testes of rats administered cadmium revealed many histopathological alterations in the form of edema, degeneration of spermatogenic cells and seminiferous tubules. In addition, cadmium induced a noticeable alteration of the spermatogenic cycle with a valuable decrease in spermatozoal production in the testis (lumen of the seminiferous tubules sections (Fig. 5 d). Additionally, the testes of animals were given (CdCl<sub>2</sub>+distilled water) showed slight enhancement of spermatogenic cells but degeneration of seminiferous tubules was observed. (Fig. 5e). In contrast, these histopathological changes were abrogated in the rats treated with (CdCl<sub>2</sub>+ StemEnhance®) and testes returned with normal architecture (Fig. 5 f).

#### **6-Electron Microscopic Examination:**

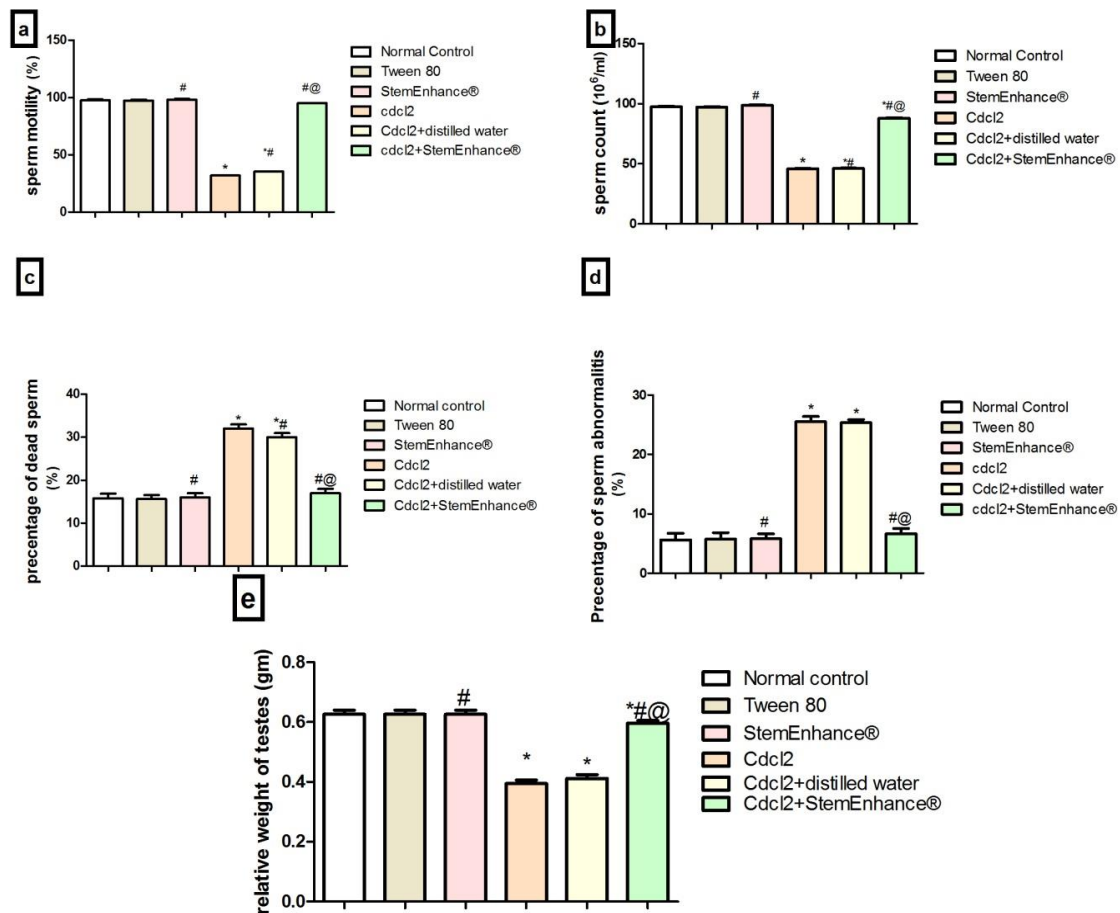
Testis of rats in normal control, tween<sup>80</sup> and StemEnhance® groups have normal seminiferous tubules and arranged Sertoli cells which have normal euchromatic nuclei with clear nucleoli. Numerous mitochondria are present in the cytoplasm. Near to basement membrane, Spermatogonia appeared with its rounded nuclei, found before primary spermatocyte that are larger in size and round in shape with large rounded nuclei. The cytoplasm of this primary spermatocyte was electron lucent with a moderate number of mitochondria (Fig. 6 a, b & c). Cadmium chloride treated group testis showed tubular affections; distorted mitochondria are found in Sertoli cells lined with some seminiferous tubules. These Sertoli cells contain dense electron bodies, intracellular vacuoles, and irregular and shrinkage nuclear envelopes in their cytoplasm. Primary spermatocyte had a disintegrated nucleus. Spermatogonium which has irregularly outlined nuclei and surrounded the peripheral heterochromatin have seen (Fig. 6d). In the testes of the rats administered (cdCl<sub>2</sub>+distilled water), some seminiferous tubules were affected. Sertoli cells demonstrated large nuclei with indented euchromatin and the cytoplasm revealed few distorted mitochondria, dense bodies and vacuoles. Also, sloughing was observed in Sertoli cell (Fig. 6e). The testes of the treated rats in the group that administrated (cdCl<sub>2</sub>+StemEnhance®) had prominent improvement. A lot of seminiferous tubules were in a normal structure. Sertoli cells had indented nuclei that rested irregularly at the basement membrane and contain mitochondria in their cytoplasm. Primary spermatocytes contained large nuclei with electron-dense chromatin masses (Fig. 6f). Spermatids in the groups (normal control, tween<sup>80</sup> and StemEnhance®) appeared with rounded nuclei (scattered chromatin), prominent (Golgi apparatus and acrosomal cap), mitochondria were arranged peripherally in the cytoplasm (Fig. 7 a, b & c). Testes of treated rats with cadmium showed Apoptotic nucleoli (shrunken nuclear envelope) in some spermatids as well as others showed a few abnormal mitochondrial distributions in their cytoplasm with dilated vacuolated intercellular spaces (Fig. 7d). Spermatids in the testes of the rats administered (cdCl<sub>2</sub>+ distilled water) appeared with pyknotic nuclei and no acrosomal cap was observed, as well as distorted mitochondria are found in their cytoplasm (Fig. 7e). In contrast, StemEnhance® treated groups showed the spermatid has round euchromatic nuclei with prominent acrosomal caps, and peripherally arranged mitochondria in the cytoplasm (Fig. 7f).

Longitudinal sections in sperms of normal control, tween<sup>80</sup> and StemEnhance® groups show normal structures. (Fig. 8 a,b,c), while in cadmium-treated rats, the sperms' tails are disorganized and have swollen mitochondria. (Fig. 8 d). Longitudinal sections in the sperms of (cdCl<sub>2</sub>+ distilled water) group demonstrated a disorganized tail of sperms (Fig.

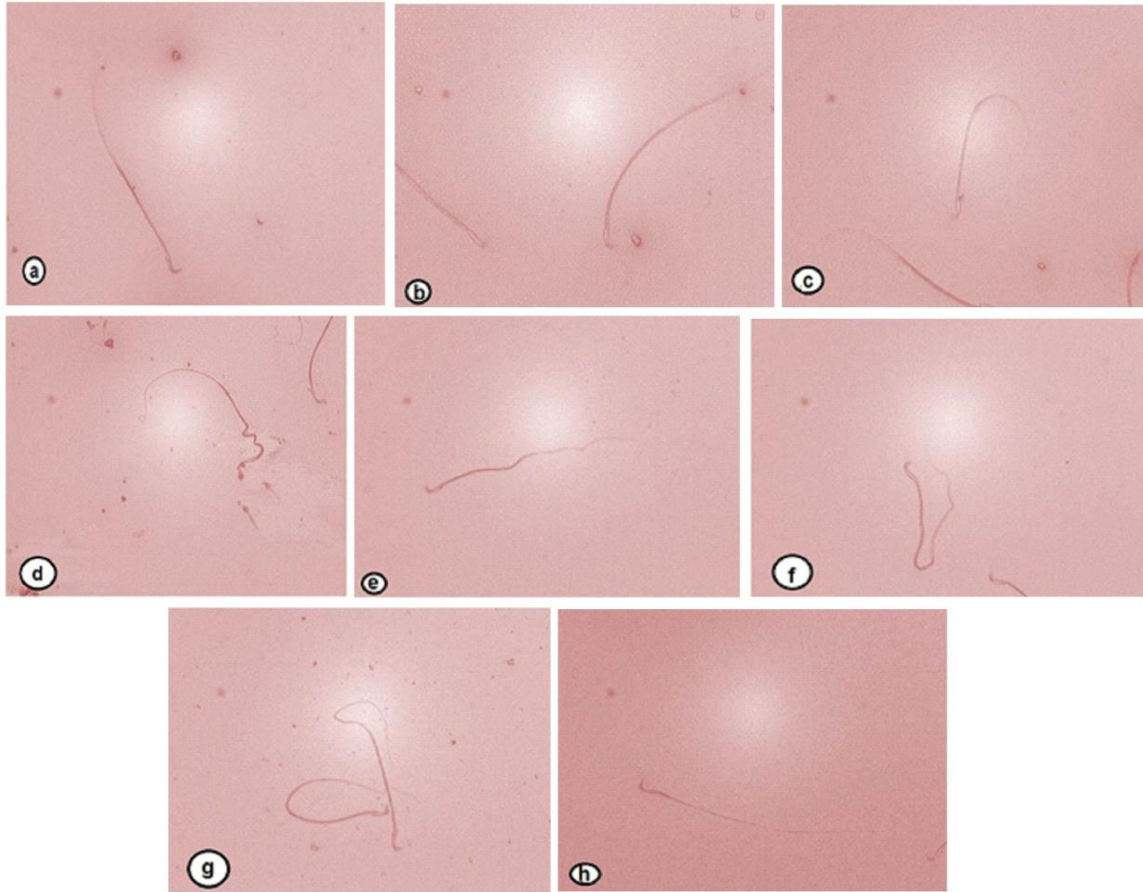


8 e), but the group that administered StemEnhance<sup>®</sup> for 45 days after cadmium group showed an improving longitudinal section of sperm. (Fig. 8 f).

The middle pieces of the spermatozoa in the transverse sections of control, tween<sup>80</sup> and StemEnhance<sup>®</sup> groups had central axoneme and mitochondrial sheath (Fig. 9 a,b,c). Cadmium treated group had disorganization of fibrous sheaths (outer sheath) and in axonemes, as well as it was surrounded by a distorted and swollen mitochondrial sheath (Fig. 9d). Testes of the rats that were treated with (cdcl<sub>2</sub>+ distilled water) had outer fibrous sheaths and swollen mitochondrial sheaths and loss of organelles in their cytoplasm (Fig. 9e). the testes of the last group which were treated by StemEnhance<sup>®</sup> for 45 days after cadmium restored the normal appearance, central axoneme as well as normal mitochondrial sheath and cell membrane (Fig. 9f).

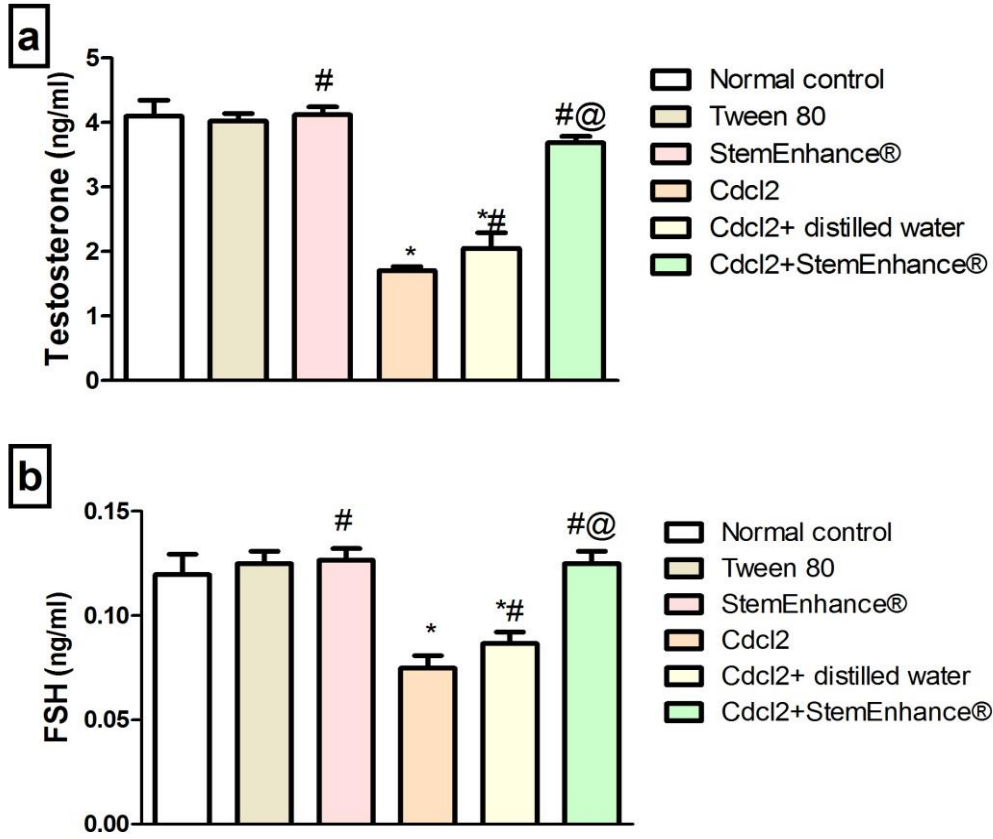


**Fig.1:** Effect of StemEnhance<sup>®</sup> administration on (a) sperm motility, (b)sperm count, (c) sperm vitality, (d)sperm abnormalities and (e) relative weight of testes in cadmium chloride-treated rats. Values are presented as mean  $\pm$  SE. \*, # or @ Statistically significant from control, cadmium chloride, or Cdcl<sub>2</sub>+distilled water groups, respectively using (ANOVA) one-way analysis of variance followed by Tukey-Kramer as a posthoc test.

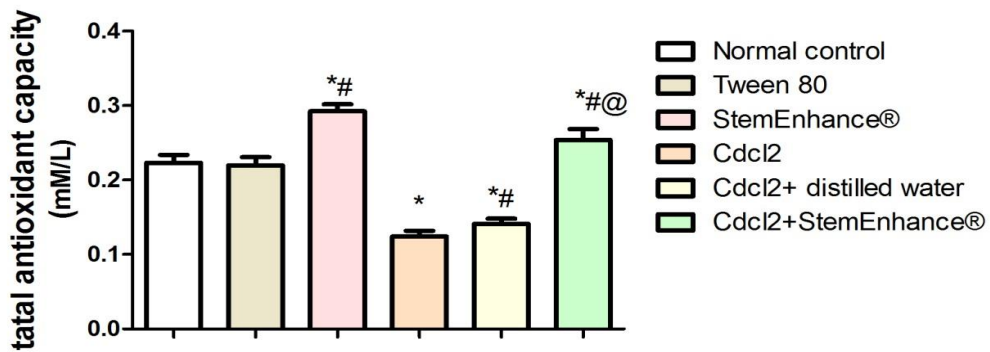


**Fig .2:** Photograph of smear from the seminal fluid of (a) control (normal) (b) tween 80 and(c) StemEnhance® groups rats showed normal sperm formation (head, neck and tail). , rat intoxicated with cadmium showing (d) twisted middle piece with amorphous head and (e) zigzag middle piece. , rat given (cdcl<sub>2</sub>+ distilled water) showing (f) bent tail and (g) coiled tail .and (h) of rat treated with (cdcl<sub>2</sub>+ StemEnhance®) normal sperm morphology with head neck and tail.

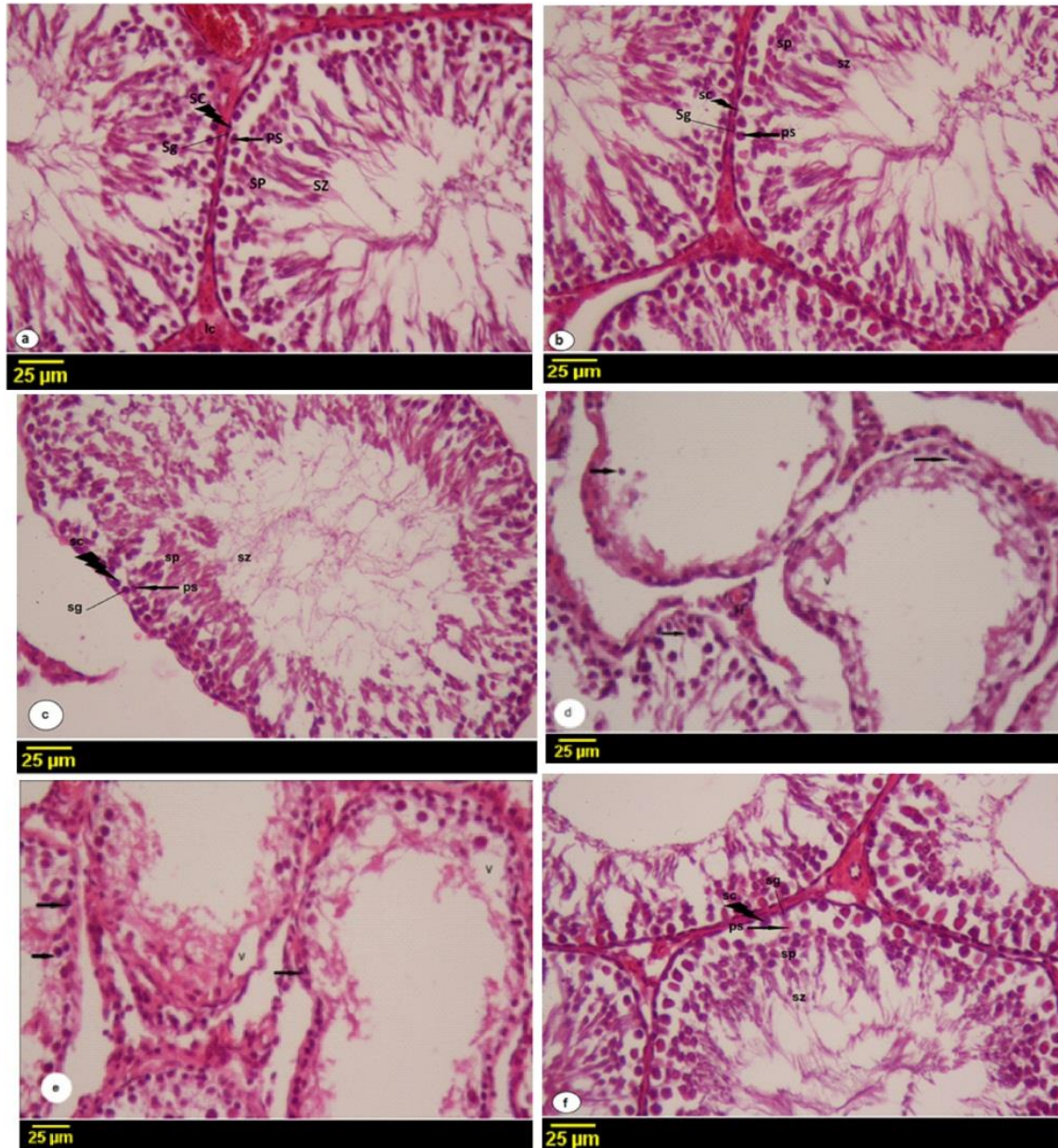




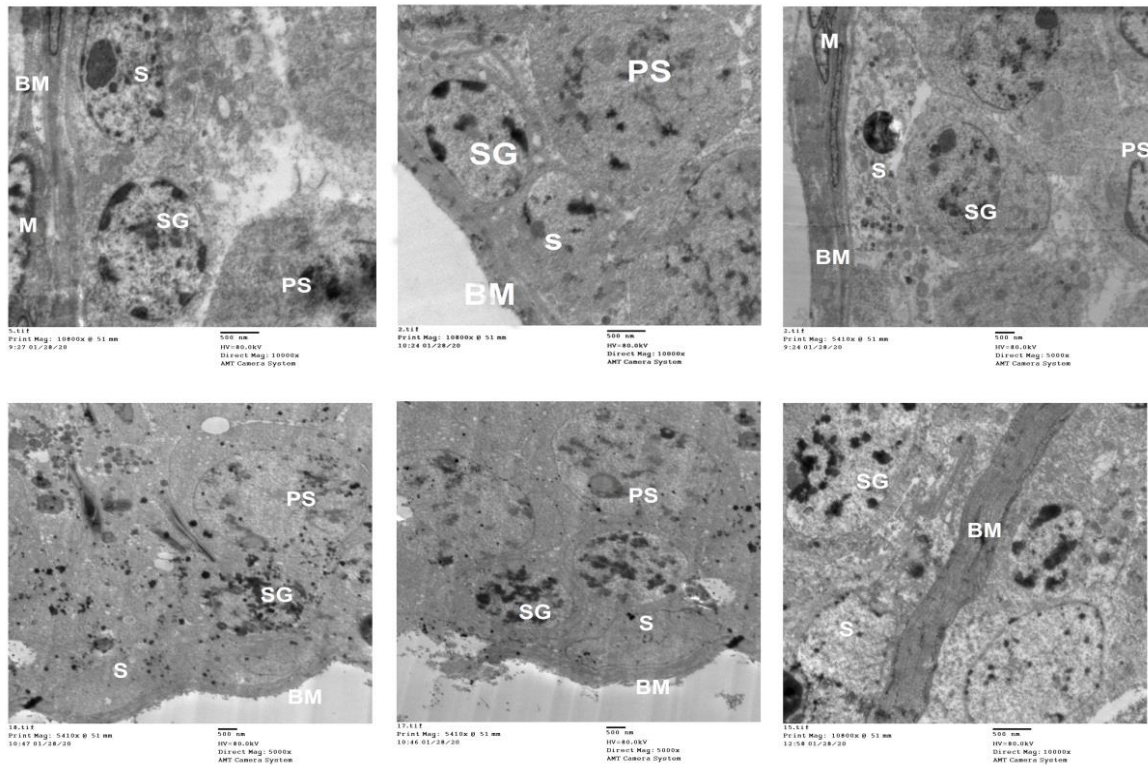
**Fig. 3:** Effect of StemEnhance®administration on (a) testosterone and (b) FSH in cadmium chloride treated rats. Values are presented as mean ± SE. \*, # or @ Statistically significant from control, cadmium, or Cdcl2+distilled water groups, respectively using one-way analysis of variance (ANOVA) followed by Tukey-Kramer as a post-hoc test.



**Fig. 4:** Effect of StemEnhance®administration on total antioxidant capacity in cadmium chloride treated rats. Values are presented as mean ± SE. \*, # or @ Statistically significant from control, cadmium, or Cdcl2+distilled water groups, respectively using one-way analysis of variance (ANOVA) followed by Tukey-Kramer as a post-hoc test.

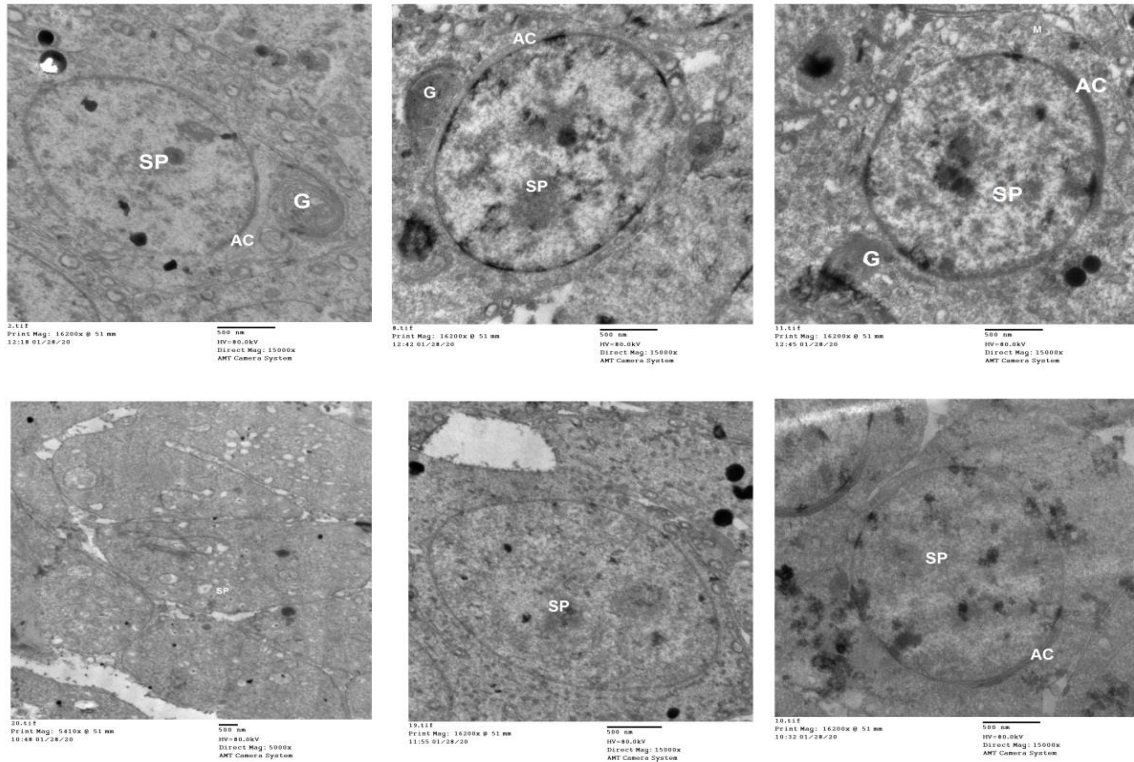


**Fig. 5:** Cross section in the testis stained with H&E (x 400) of the rat. **(a)** normal control, **(b)** tween<sup>80</sup> and **(c)** StemEnhance® groups showing normal spermatogenic cells, spermatogonia (sg), primary spermatocyte (ps), spermatid (sp), spermatozoa (sz) and Sertoli cells (sc). **(d)** Treated with CdCl<sub>2</sub> showing Degeneration of spermatogenic cells, pyknotic nuclei (arrows), and intercellular vacuoles (v). **(e)** treated with (CdCl<sub>2</sub> +distilled water) showing degeneration of spermatogenic cells, pyknotic nuclei of some spermatogenic cells (arrows) **(f)** treated with (CdCl<sub>2</sub>+StemEnhance®) showing complete healing of spermatogenic cells in most seminiferous tubules.

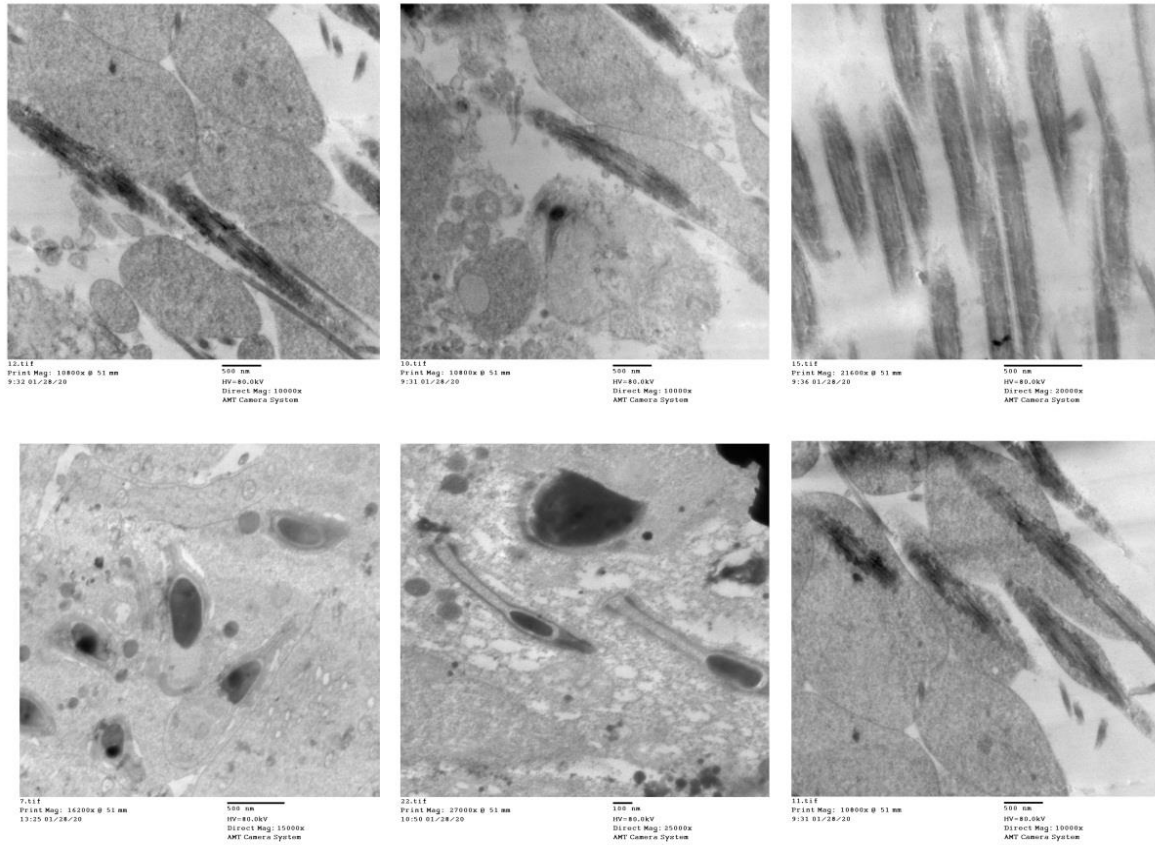


**Fig. 6:** electron photomicrographs of adult albino rat testis (ultrathin sections) (a) normal control (b) tween<sup>80</sup> and (c) StemEnhance® groups showing Sertoli cell (S), Spermatogonium (SG) and primary spermatocyte (PS) has rounded nuclei lying on a basement membrane (BM) regular with myoid cell (M). (d) Treated with cadmium showed Sertoli cell (S) with an irregular shape, Spermatogonium (SG) with pyknotic nuclei and primary spermatocyte with pyknotic nuclei are seen lying on irregular and rupture basement membrane (BM) and absence myoid cell. (e) Treated with (CdCl<sub>2</sub> +distilled water) showing Spermatogonium (SG) with pyknotic nuclei and primary spermatocyte with pyknotic nuclei are lying on irregular basement membrane (BM) and absence myoid cell. (f) Treated with (CdCl<sub>2</sub> + StemEnhance®) showing improving Sertoli cell (S), Spermatogonium (SG) and primary spermatocyte with rounded nuclei are seen lying on a mild irregular basement membrane (BM).

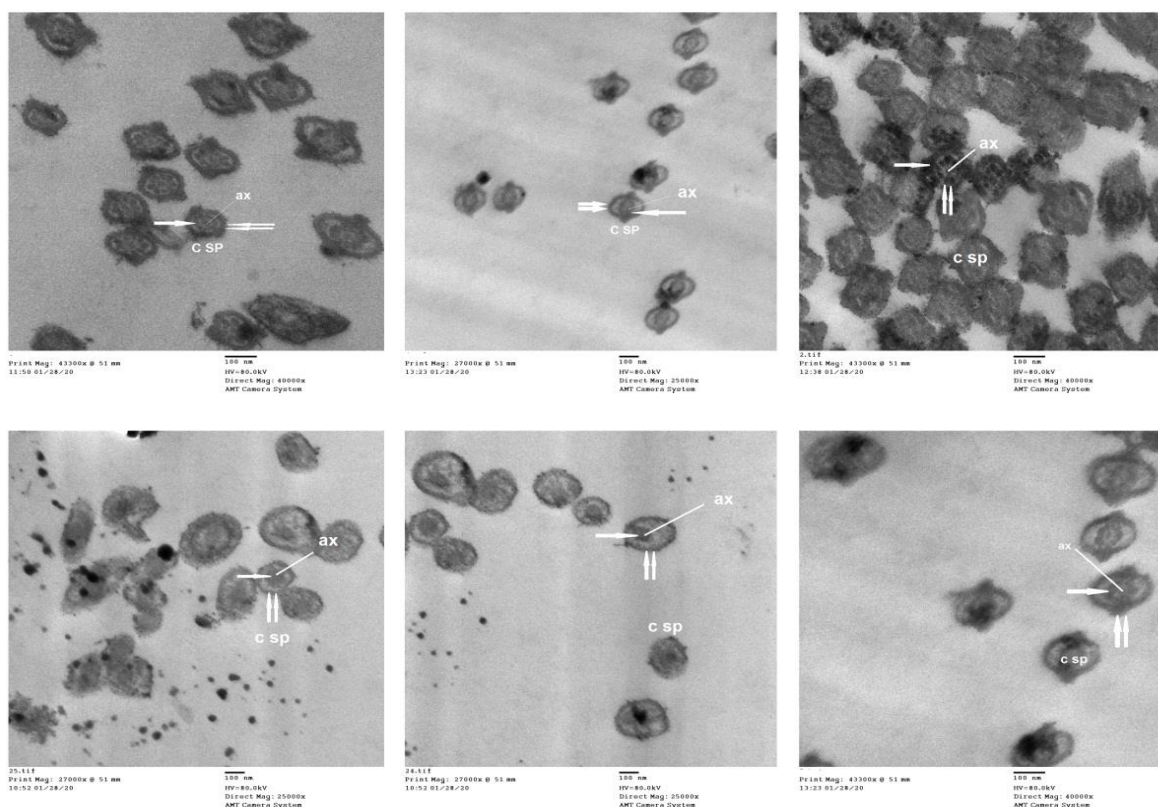




**Fig. 7:** photomicrographs of adult albino rat testis (ultrathin section) (a) normal control (b) tween<sup>80</sup> and (c) StemEnhance® showing normal spermatids (Sp) with large round euchromatic nuclei. At the nucleus side, the Golgi apparatus (G) and acrosomal cap (AC) are present (on one side). The cytoplasm had mitochondria (m, peripherally arranged ). (d) treated with cadmium showed spermatids (Sp) which had an Apoptotic nucleus (shrunken nuclear envelope) and the absence of an acrosomal cap and golgi apparatus. The spermatid cytoplasm contains shrunken mitochondria and vacuoles between spermatids. (e): treated with (CdCl<sub>2</sub> +distilled water) showing spermatids (Sp) with the shrunken nuclear envelope (Apoptotic nucleus). The cytoplasm of the spermatid contains shrunken mitochondria and an absence of an acrosomal cap. (f): treated with (CdCl<sub>2</sub> + StemEnhance®) showed improvement in spermatids (Sp) containing rounded euchromatic nuclei. Golgi apparatus (G) and acrosomal cap (AC) at one side of the nucleus are present.



**Fig. 8:** photomicrographs of an ultrathin section of adult albino rat testis **(a)** normal control **(b)** tween <sup>80</sup> and **(c)** StemEnhance® showing a normal longitudinal section in sperm (E sp). **(d):** treated with cadmium showing disorganization longitudinal sections in sperm. **(e):** treated with (CdCl<sub>2</sub> + distilled water) showing longitudinal sections of sperm as tails of sperm (mid or principal pieces) which still showed disorganization. **(f):** treated with (CdCl<sub>2</sub> + StemEnhance®) showing a normal longitudinal section of sperms.



**Fig. 9:** photomicrographs of adult albino rat testis (ultrathin section) (a) normal control (b) tween<sup>80</sup> and (c) StemEnhance® showed cross sections in spermatozoal middle pieces of the axoneme (ax) surrounded with nine outer dense fibers (arrow) and cell membrane (double arrows). (d) Treated with cadmium showed a cross-section in sperm tail has disorganization of the axonemes. (e): treated with (cdcl<sub>2</sub>+ distilled water) showed cross sections in sperm tails with disorganization of the axonemes. (f): treated with (cdcl<sub>2</sub>+ StemEnhance®) cross sections in the middle pieces of the spermatozoa. In the mid pieces, the axoneme (ax) is surrounded by nine outer dense fibers (arrow), and a cell membrane (double arrows).

## DISCUSSION

One of the contaminants that affect numerous tissues and organs especially the testicles is cadmium (Cd) (Aktas *et al.*, 2012) causing testicular damage and sperm toxicity (Mudathir *et al.*, 2008).

Herein, we tried to find the possible treatment effect of StemEnhane® against CdCl<sub>2</sub>-induced testicular damage in rats.

Excessive release of reactive oxygen species (ROS) or insufficiency of antioxidants is known as oxidative stress (OS) which is considered a main cause of sperm damage and male infertility (Agarwal *et al.*, 2018) and causes inflammatory responses in the male genital system causing oxidative damage to reproductive cells and intracellular components (Dutta *et al.*, 2021).

Mechanisms by which Cd-induced organ toxicity are oxidative stress, inflammation, apoptotic cell death and chromosomal aberrations (Bashir *et al.*, 2019 and Kaur *et al.*, 2020) as oxidative stress plays an important role in cadmium toxicity which cause physiological damage to many organs as reproductive organs (Rani *et al.*, 2013).

One of the most important diagnostic tests used in the male infertility workup is the determination of total antioxidant capacity (TAC) as TAC evaluates the level of total antioxidants in seminal plasma fluid (Gupta *et al.*, 2021). In our study, cadmium chloride-



induced downregulation of total antioxidant capacity and this result is similar to those obtained by (Wang *et al.*, 2021) and this duo to mechanisms involved in cadmium can increase oxidative stress which is lipid peroxidation and antioxidant depletion (Arafa *et al.*, 2014) and generation of free radicals (Whittaker *et al.*, 2004). On the other side, co-treatment with StemEnhane® upregulated total antioxidant capacity and this may be attributed to its antioxidant effect which can effectively mitigate oxidative stress (Helal *et al.*, 2019).

To confirm the toxicity of CdCl<sub>2</sub>, ultrastructure examination (by the electron microscopic) was performed to show the degree of affection on tubular and Sertoli cells with distorted mitochondria, intracellular vacuoles and irregular nuclear envelope. these cellular changes are similar to those of (Arafa *et al.*, 2014). Also, we found intercellular spaces with sometimes vacuoles caused indentation of the near germ cells, this finding is in the same line with (Labib and Galal 2020) and these affected germ cells are the main factor of sperm abnormalities (Acharya *et al.*, 2003). There is a disintegrated nucleus for both primary spermatocyte and Spermatogonium (irregular outline nucleus), also spermatids had shrunken nuclear membranes with abnormal distribution for the mitochondria in the cytoplasm and dilated intercellular spaces (contain vacuoles) (Haffor and Abou-Tarboush 2004; Niknafs *et al.*, 2015). Also, electron microscope examination cleared that, the sperm appeared with a disorganized tail, swollen mitochondria in the middle pieces due to Cadmium which cause disorganization of axonemes and the outer fibrous sheaths and also swollen and distorted mitochondrial sheath. This important deep effect of cadmium is counteracted by using StemEnhane® as all cells return normally and this is attributed to its ability to mobilize adult bone marrow stem cells (Dirikolu *et al.*, 2010) which can proliferate, regenerate, and transform into different cells with different functions (Abdelaziz *et al.*, 2019).

Organs' weight is considered a valuable toxicological indicator. Our results recorded a reduction in the relative weight of the testes after treatment with CdCl<sub>2</sub> this result agreed with a previous study by (Arafa *et al.*, 2014). The loss of testes' weight due to Cd occurred in the form of reduced tubule size, spermatogenic arrest, and the inhibition of biosynthesis for steroids in Leydig cells (Bashir *et al.*, 2019).

This effect was reversed by StemEnhane® which restored the relative weight of the testes. The mechanism of this amelioration is its ability to stimulate the mobilization of bone marrow stem cells which stimulates tissue regeneration for a lot of animals model (Drapeau *et al.*, 2010).

In our study Cadmium chloride caused significant decrease in testosterone and FSH levels and this agreed with (Hachfi and Sakly 2009 ; Zhang *et al.*, 2017) who mentioned that decrease in FSH level is a sign of cadmium toxicity, this effect is due to the fact that cadmium alters the regulatory mechanisms of hypothalamic–pituitary–gonadal axis by modifying neurotransmitters responsible for this regulation at the hypothalamic level, affecting gonadotropin hormone secretion and by its harmful effect on testicular structure and activity (Lafuente 2013) Normally, the hypothalamus produces gonadotrophin-releasing hormone (GnRH) which stimulates the pituitary for LH synthesis which regulate Leydig cell function but cadmium intoxication negatively affects testicular function by decreasing pituitary LH release and diminishing Leydig cell steroidogenesis (Farombi *et al.*, 2012) or may be due to decreasing steroidogenic enzymes 3β-HSD and 17β-HSD which are responsible for testicular androgenesis (Gupta *et al.*, 2004). Treatment with StemEnhane® halted the toxic effect induced by cadmium chloride by upregulation of testosterone hormone and this duo to the ability of StemEnhane® to modulate serum hormones (Helal *et al.*, 2019<sup>(a)</sup>).

In this investigation, Cadmium chloride caused a decrease in sperm count and this result was confirmed by (Abdelrazek *et al.*, 2016), the cause of this result is the decrease in FSH levels which is observed in Cd-exposed animals as any decrease in FSH and testosterone level leads to a decrease in the sperm count (Hachfi and Sakly 2009; Helal *et al.*

., 2019<sup>(b)</sup>). On the contrary, low sperm count in cadmium-treated groups was increased by using StemEnhance® by increasing FSH and testosterone as FSH and testosterone are involved as synergistic in the process of spermatogenesis (Helal *et al.*, 2019<sup>(b)</sup>).

Also, cadmium caused a decrease in sperm motility and increased sperm abnormalities, our data on epididymal sperm count, motility and abnormalities were in accordance with previous reports by (Bashir *et al.*, 2019; Zhang *et al.*, 2017) who found that treatment by Cd reduced the sperm motility and increased the abnormal sperm rate. This is attributed to the increase in ROS release and decreasing different antioxidants levels by cadmium which enhance the lipid peroxidation of cell membranes causing apoptosis and necrosis of all testicular tissue resulting in disturbance of spermatogenesis and thus decrease sperm motility and finally leading to infertility (Alaee *et al.*, 2014) as normally mature sperms highly contain polyunsaturated fatty acids in which the oxidative stress occurs so Cd intoxication enhances the sperm membrane lipid peroxidation which decreases sperm motility and increases the abnormal sperm percent as well as leading to decrease in the fertilizing capacity of sperm (Bashir *et al.*, 2019) and also the affection of germ cells which appeared in our ultra-structure examination by electron microscope is a main cause as damaging the germ cells leading to a significant decrease in sperm count and production of varieties of abnormal sperm (Acharya *et al.*, 2003). StemEnhance® enhanced the sperm profile as mentioned in previous studies as mesenchymal stem cells (MSCs) mobilised by StemEnhance® improve sperm function mainly by the augmentation of sperm motility and energy (Hsiao *et al.*, 2019).

Our biochemical findings were confirmed by histopathological findings where cadmium caused testicular tissue alteration in the form of edema, degeneration of spermatogenic cells, pyknotic nuclei, dilatation and congestion of blood vessels. Additionally, it caused an alteration of the spermatogenic cycle with reductions in spermatozoal production. Previous studies by (Zhang *et al.*, 2017) demonstrated that Cd toxicity resulted in vacuole degeneration, apoptosis and necrosis in the seminiferous epithelium and degenerative seminiferous tubules (Almeer *et al.*, 2018), congestion of interstitial vessels and oedema in cadmium-treated testes (Farombi *et al.*, 2012). These confirmed our results as this may be due to a direct or indirect effect of ROS that stimulate lipid peroxidation which is a chemical mechanism that can alter the function and structure of the organ (Farombi *et al.*, 2012). These histopathological alterations were hampered by StemEnhance® which is a bone marrow stem cell mobilizer as it stimulated the mobilization of adult stem cells from the bone marrow (Ismail *et al.*, 2013) and these adult bone marrow (BM)-derived stem cells including HSCs (hematopoietic stem cells) and MSCs (mesenchymal stem cells) are a valuable source of cells to repair the number of damaged tissues (Ponte *et al.* 2007) also may be due to its antioxidant effect which reduces lipid peroxidation by upregulating of cellular antioxidants (Liu *et al.* 2010).

### **Conclusion**

In conclusion, this investigation revealed that StemEnhance® treatment abrogated CdCl<sub>2</sub>-induced testicular injury in rats by enhancing spermatogenesis, decreasing oxidative stress damage and due to its action as a stem cell mobilizer. We suggest that future studies should focus on the use of StemEnhance® as a treatment for reproductive damage caused by heavy metals.

### **Compliance and Ethical Standards:**

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Ethical Approval:** All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

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**Author Contribution:** K.A: Conceptualization, Methodology, Validation, Formal analysis,

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