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RESEARCH ARTICLE

Evaluating the ameliorative efficacy of *Spirulina platensis* on spermatogenesis and steroidogenesis in cadmium-intoxicated rats

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Abstract The present study was conducted to evaluate the ameliorative efficacy of *Spirulina platensis* (SP) on reproductive dysfunctions induced by cadmium chloride ($CdCl_2$) in male rats. Rats (n=40) were divided into five groups (eight rats/each). Group 1: served as control without any treatment. Group 2: Rats were administered SP (150 mg/kg body weight (BW)) in drinking water for 10 days. Group3: Rats were subcutaneously injected with $CdCl_2$ (2 mg/kg BW) daily for 10 days. Group 4: Rats were co-treated with both $CdCl_2$ (2 mg/kg BW) and SP (150 mg/kg BW) daily for 10 days (SP prophylactic group). Group5: Rats received $CdCl_2$ for 10 days followed by administration of SP alone in drinking water daily for another 30 days with the same mentioned routes and doses (SP treatment group). From our findings, the administration of SP alone or co-administration with Cd significantly attenuated the harmful effects of Cd, suggesting its beneficial role in improving spermatogenesis and steroidogenesis after Cd exposure.

Keywords Cadmium · Spermatogenesis · Steroidogenesis · *Spirulina platensis* · Rat

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Abbreviations

$CdCl_2$	Cadmium chloride
SP	*Spirulina platensis* powder
TES	Testosterone
LH	Leutinizing hormone
EST	Estradiol
17β-HSD3	17β-Hydroxysteroid dehydrogenase type 3
3β-HSD6	3β-Hydroxysteroid dehydrogenase type 6
NR5A1	Steroidogenic factor 1 (SF1)

Introduction

The adverse health effects related to the reproductive dysfunction and male infertility have become a source of greatest concern worldwide. About half of the male infertility cases in humans of unknown origin suggest the involvement of physiological disorders or due to occupational or environmental exposures as many of these factors have been implicated to determine suboptimal quality of semen and to reduce reproductive hormone production (Patra et al. 2011).

Cadmium (Cd) is a toxic metal present in the environment either naturally or as a contaminant arising from different sources including agricultural and industrial ones (Järup and Äkesson 2009). Acute and chronic Cd toxicity have been reported to result in a variety of biochemical and physiological disorders due to extensive injury to different body organs both in humans and laboratory animals (Gaurav et al. 2010). Studies have demonstrated that the testis is highly sensitive to toxicity with cadmium (Liu et al. 2009), manifested by decline in male fertility parameters, such as decreased count of spermatozoa and poor semen quality (Wafaa et al. 2012; Ekhoye et al. 2013). The adverse impacts of Cd on male fertility come through its capability to induce degenerative changes in components of reproductive system (testes, epididymis, and

seminal vesicles as well as Leydig cells), promoting an inhibition of steroidogenesis and generation of reactive oxygen species that lead to cell necrosis and apoptosis (Rennolds et al. 2012; Nair et al. 2013).

Currently, spirulina, a microscopic multi-cellular filamentous blue green algae (Cyanobacterium) known as a "super food" is gaining the attention of medical scientists due to the ability in protecting the body physiological system against oxidative damage as well as nutraceutical and source of potential pharmaceuticals. Therefore, its long history of safe use makes it a unique blue green algae (Sheshadri and Umesh 1992). It has been used as a nutritional supplement for human and animal consumption as it is rich in proteins, lipids, carbohydrates, sterols, and some essential elements such as zinc, magnesium, and selenium (Babadzhanov et al. 2004). *Spirulina platensis* (*Arthrospira platensis*) is a natural source of vitamins, macronutrients and micronutrients like amino acids, gamma linolenic acid, carotenoids, especially β-carotene, α-linolenic acid, phycocyanin and phycocyanobil in chlorophyll, and xanthophyll phytopigments (Upasani and Balaraman 2003; Gong et al. 2005; Bermejo et al. 2008). The administration of *Arthrospira* produced a significant protective effects against oxidative damage caused by Cd in liver and kidney of rats (Jeyaprakash and Chinnaswamy 2005), nephrotoxicity of mercury in mice (Sharma et al. 2007) as well as a protective role in the oxidative stress in nervous system of offspring from rats exposed to fluoride (Banjia et al. 2013) and enhance the spermatogenesis and steroidogenesis in diabetic rats (Nah et al. 2012). However, role of SP in ameliorating the reproductive dysfunctions induced by Cd is not fully established. Therefore, the present study aimed to determine whether SP could attenuate Cd-induced testicular damage, by investigating the possible antagonistic actions of SP on several aspects of testicular dysfunctions, biochemical and pathological abnormalities in male rat testes induced by cadmium chloride ($CdCl_2$), as well as the expressions of some steroidogenic genes in testicular tissues.

Material and methods

Animals

Healthy adult male Sprague–Dawley rats (n=40 and average body weight of 180–200 g) were used in this study. They were obtained from the Laboratory Animal Housing Unit, Research, Institute, Dokki, Cairo, Egypt. The animals were clinically healthy and kept under hygienic conditions in stainless steel cages with hard wood shavings as bedding. They were maintained on basal ration, given water ad libitum and exposed to 12 h light-darkness cycle for 2 weeks of acclimatization before use.

Tested compounds

$CdCl_2$ was procured from El-Faraana Co. for Trading, Egypt, in the form of white powder. *Spirulina platensis* (SP) was procured from Free Trade Egypt Co., Behira, Egypt.

Experimental design

The 40 experimental rats were assigned into five equal groups each containing eight rats (with nearly equal body weight (BW)). Group 1 was kept as control and received no treatments. Group 2 was administered SP (150 mg/kg BW) in drinking water using water bottle for 10 days. The body weights of rats were measured every other day, and the amount of SP in drinking water (150 ml) was newly adjusted to 150 mg/kg BW. Group 3 was given $CdCl_2$ (2 mg/kg BW) by subcutaneous injection (S/C) daily for 10 days. Group 4: Rats were co-treated with both $CdCl_2$ (2 mg/kg BW) and SP (150 mg/kg BW) daily for 10 days (SP prophylactic group). Group 5: Rats were received $CdCl_2$ for 10 days and then the cadmium administration was stopped and the same rats continued to receive SP alone in drinking water daily for another 30 days at the same mentioned routes and doses (SP treatment group).

Sample collection and preparation

At the end of the experiment, the animals were sacrificed followed by collection of blood without anticoagulant, then centrifuged at 3000 rpm for 10 min for separation of serum which was stored at −20 °C for hormonal analysis (determination of testosterone (TES), leutinizing hormone (LH), and estradiol (EST) levels). After dissection, the testicles were removed, trimmed off the attached tissues, grossly examined, and weighed. Tissue samples of testes were rapidly removed from depicted rats, quickly kept in liquid nitrogen until RNA extraction for determination of 3β-hydroxysteroid dehydrogenase type 6 (3β-HSD6), 17β-hydroxysteroid dehydrogenase type 3 (17β-HSD3) and Nr5A1 (=steroidogenic factor 1 (SF1)) expression level using a semi-quantitative real-time RT-PCR. Tails of epididymes were excised immediately for semen evaluation. Other samples from testes, seminal vesicles, and prostate glands of both control and treated animals were fixed in 10 % neutral buffered formalin for histopathological examination.

Reproductive organ weights

Testes were excised, and the epididymes were carefully removed from them. Then, testes weights were recorded; similarly, the seminal vesicles were excised from prostates, and their weights were taken.

Semen evaluation (epididymal spermatozoal examination)

The cauda epididymis of one testis was excised and received in a sterilized petri dish containing warm normal saline at 37 °C, and then, it was macerated by sterilized scissors to obtain the epidydimal contents in a suspension that was handled as the semen (Wales 1971). The percent of motile spermatozoa was microscopically estimated at 400× magnification (sperm motility) as described by Slott et al. (1991), and sperm cell concentration per milliliter of semen was performed according to the method of Robb et al. (1978); the count was repeated five times for each sample to minimize the error. Sperm abnormalities were recorded using the method of Filler (1993). To assess the incidence of abnormalities in head, neck/mid-piece, and tail, at least 500 spermatozoa were observed per animal.

Hormonal assays

Serum hormonal TES, LH, and EST levels were determined using an enzyme-linked immunosorbent assay (ELISA) with commercial kits, following manufacturer's instructions. Serum TES and estradiol were evaluated using Oxis International, Inc., USA, Catalog Nos. 11150 and 11110 kits, respectively. The sensitivity of assays were 0.05 ng/ml and 10 pg/ml, respectively, and the level expressed respectively as ng/ml and pg/ml. Serum LH was quantified using BioCheck, Inc., USA, Catalog No. BC-1031 kit; the sensitivity of the assay was 1 mIU/ml.

Analysis of gene expressions by real-time RT-PCR in testicular tissues

To evaluate the effects of cadmium chloride on the steroidogenesis in Leydig cells, testicular messenger RNA (mRNA) levels of the steroidogenic pathway genes 17β-HSD3, 3β-HSD6, and NR5A1 were analyzed by semi-quantitative real-time RT-PCR according to Meadus (2003). Briefly, total RNA was isolated from testes samples using Qiagen RNA extraction kits, (Cat. No. 74104). The amount of extracted RNA was quantified and qualified using NanoDrop® ND-1000 Spectrophotometer, NanoDrop Technologies, Wilmington, Delaware, USA. The purity of RNA was checked and ranged between 1.8 and 2.1, demonstrating the high quality of the RNA. The mRNA was stored at −20 °C before RT-PCR. RNA was reversed for production of complementary DNA (cDNA) using QIAGEN Long Range 2 Step RT-PCR Kit, (Cat. No.205920). One microliter of total cDNA was mixed with 12.5 μl of 2× SYBR® Green PCR mix with ROX from Bio-Rad, 5.5 μl of autoclaved water, and 0.5 μl (10 pmol/μl) of each forward and reverse primer for the measured genes. The housekeeping gene β-actin was used as a constitutive control for normalization. Primer sequences of rat 3β-HSD6, 17β-HSD3, Nr5A1, and β-actin were obtained from the published sequences of Nah et al. (2012). The primer design was optimized for RT-PCR with EugeneTM version 2.2 (Daniben Systems, Cincinnati, OH, USA) (Table 1).

Histopathological investigation

Tissue specimens from testis, seminal vesicles, and prostate glands of both control and treated animals were fixed in 10 % neutral buffered formalin, dehydrated in gradual ethanol (70–100 %), cleared in xylene, and embedded in paraffin. Five micron thick paraffin sections were prepared and then routinely stained with hematoxylin and eosin (H and E) dyes according to Bancroft and Stevens (1996).

Statistical analysis

Data were statistically analyzed using the software SPSS/PC+ (2001) for obtaining mean data and standard error. Data were analyzed using one-way ANOVA to determine the statistical significance of differences among experimental groups.

Results

Effects of $CdCl_2$, SP powder, and their co-exposure on body weight

Results presented in Table 2 show that $CdCl_2$ administration significantly decreased ($p<0.05$) the BW of exposed rats during the entire experimental period compared with the control and other treatments. The prophylactic use of SP (Cd + SP) or administration of SP alone could maintain the BW of rats similar to control value. However, using of SP as treatment (Cd then SP) could enhance the BW change of animals but still lower than control group.

Effects of $CdCl_2$, SP powder, and their co-exposure on relative weights of reproductive organs

With regards to relative testicular weight, $CdCl_2$-administered animals gained less testicular weight than the controls ($p<0.05$; Table 3). However, co-administrated groups of SP either as prophylactic or as treatment could normalize the $CdCl_2$ reducing effect, and their testicular weights were maintained at the level of the control. The relative weights of both seminal vesicle and prostate gland do not significantly changed ($p<0.05$) in all experimental groups than control.

Table 1 Oligonucleotides for semi-quantitative real-time PCR analysis

Gene		Primer sequence (5′→3′)	GenBank Acc. No.
3β-HSD6	Forward	GCATTAACCCCACTCCCACT	NM_017265
	Reverse	GGACCCTGACCTCCTTCAGA	
17β-HSD3	Forward	GTGTGCACATTTTCCAAGGC	NM_054007
	Reverse	TTTAACAAACTCATCGGCGG	
Nr5A1	Forward	CGCCAGGAGTTTGTCTGTCT	NM_001191099
	Reverse	ACCTCCACCAGGCACAATAG	
β-actin	Forward	TCACTATCGGCAATGTGCGG	NM_007393
	Reverse	GCTCAGGAGGAGCAATGATG	

Seminal picture

The counts of epididymal sperm and motility were notably decreased in $CdCl_2$-administered rats. The administration of SP alone could numerically increase the motility of the sperms (93.33±1.66) and significantly ($p<0.05$) increase their counts (74±1.28) than control group (89.00±2.88 and 69±0.66, respectively). In addition, the co-administration of SP with $CdCl_2$ (prophylactic) largely counteracted the unfavorable actions of $CdCl_2$ and was more effective in increasing the motility % (83.33±1.66) and sperm count (61±0.88) to be comparable to those of control rats than $CdCl_2$ then SP (treatment group) which showed a motility % of 76.66±3.33 where the sperm count of this group was (59.33±0.22).

The S/C injection of $CdCl_2$ to rats resulted in a significant increase ($p<0.05$) in the incidence of abnormal spermatozoa (17.50±0.29) compared to control (6.91±0.33). The prophylactic use of SP significantly decreased ($p<0.05$) the % of sperm abnormalities (8.42±0.29) than $CdCl_2$ group and gave results near to control values; the $CdCl_2$ then SP (treatment) group could also decrease the % of abnormal spermatozoa (12.04±0.31) than $CdCl_2$ group but still significantly higher than control. However, the % of abnormal spermatozoa in rats administered SP alone (6.08±0.12) was similar to those in control group (Fig. 1). As illustrated in Fig. 2, the primary pathologic spermatozoa were detached, broken, and abnormal head shape as well as detached tail, while the secondary abnormalities were coiled, curved, and looped tail and cytoplasmic droplets.

Hormonal analysis

The effects of administration of $CdCl_2$ and SP on hormonal levels of treated rats are represented in Table 4. The results showed a significant decrease in serum level of TES in the $CdCl_2$ group compared to control. The administration of SP alone showed a significant increase in serum TES level compared to control. On the other hand, $CdCl_2$ + SP group (prophylactic) and $CdCl_2$ then SP treatment group showed non-significant differences in TES level than control. The serum level of LH hormone did not significantly differ among all the experimental groups. Additionally, EST level showed non-significant differences among treated groups expect in the $CdCl_2$ then SP group where the EST level was significantly decreased than control groups.

Expressions of steroidogenic genes in testicular tissues

In $CdCl_2$-treated rat testes, steroidogenic genes' mRNA expressions were significantly downregulated compared to the control. SP intake significantly upregulated 3β-HSD6 and 17β-HSD3 to be better than control group. However, administration of SP with $CdCl_2$ (prophylactic group) significantly increased 3β-HSD6 mRNA and 17β-HSD3 levels to be similar to control values. The rats administered SP following $CdCl_2$ (treatment group) showed a significant improvement in the expression of these two genes but still lower than prophylactic group and control group values (Fig. 3). Administration of SP alone significantly upregulated the expression of the Nr5A1 mRNA; however, there were no significant

Table 2 Effect of Cd and SP (prophylactic and treatment) on body weight (BW) change of exposed rats (mean ± SE, $n=8$)

Items	Initial body weight (g)	Final body weight (g)	Body weight change (g)
Control	186.66±6.66	226.66±3.33^{a}	40.00±5.77^{a}
SP	187.00±3.60	233.33±4.40^{a}	47.00±1.00^{a}
$CdCl_2$	186.66±2.96	160.33±5.17^{c}	−26.33±2.60^{c}
$CdCl_2$ + SP	187.66±1.45	199.00±2.30^{b}	11.33±0.88^{b}
$CdCl_2$ then SP	187.66±2.84	220.33±6.64^{a}	31.66±8.19^{a}

Means within the same column carrying different superscripts are significantly different ($p\leq0.05$)

Table 3 Effect of Cd and SP (prophylactic and treatment) on relative weight of reproductive organs of exposed rats (mean ± SE, n=8)

Items	Relative testes weight (g)	Relative seminal vesicle weight (g)	Relative prostate weight (g)
Control	0.94±0.99^{ab}	0.85±0.07	0.37±0.03
SP	0.96±0.01^{a}	0.90±0.06	0.43±0.04
$CdCl_2$	0.79±0.01^{c}	0.72±0.11	0.28±0.02
$CdCl_2$ + SP	0.88±0.31^{ab}	0.75±0.04	0.34±0.04
$CdCl_2$ then SP	0.82±0.01^{ab}	0.84±0.06	0.36±0.05

Means within the same column carrying different superscripts are significantly different ($p \leq 0.05$)

differences in its level among the all other treatment groups compared to control.

Histopathological findings

Testes

The testes of control or SP-received rats showed uniform seminiferous tubules with complete spermatogenesis and interstitial connective tissue. The tubular epithelium was highly intact and contained Sertoli cells resting on the basement membrane, together with spermatocytes and spermatogonia. Round and elongated spermatids were embedded in or associated with the Sertoli cells at different stages of the spermatogenic cycle (Fig. 4a 1). While, the testes of cadmium-received group showed testicular degeneration that was represented by severe vacuolation (Fig. 4a 2), desquamation, and necrosis (Fig. 4a 3) in the germinal epithelium of the seminiferous

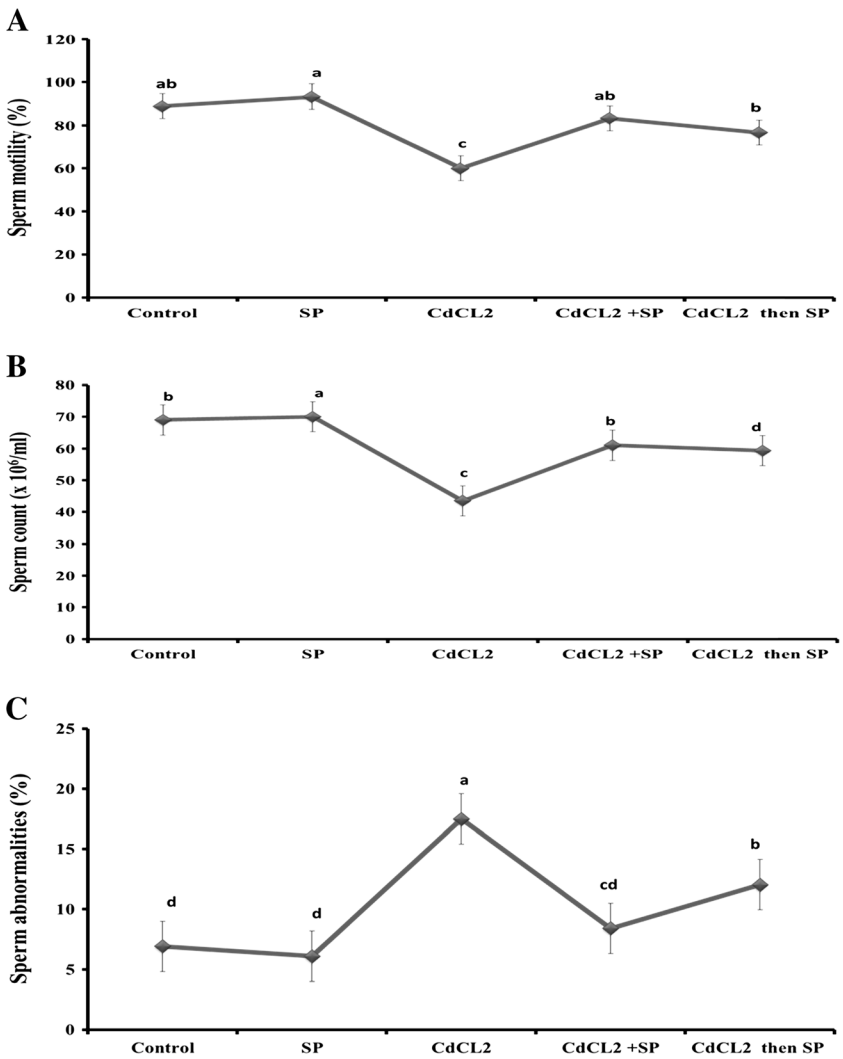

Fig. 1 Effect of $CdCl_2$ and SP and their co-exposure on sperm characteristics of treated rats. Data expressed as mean ± SE (n=8 replicates). *Columns carrying different superscripts* are significantly different (one-way ANOVA) ($p \leq 0.05$)

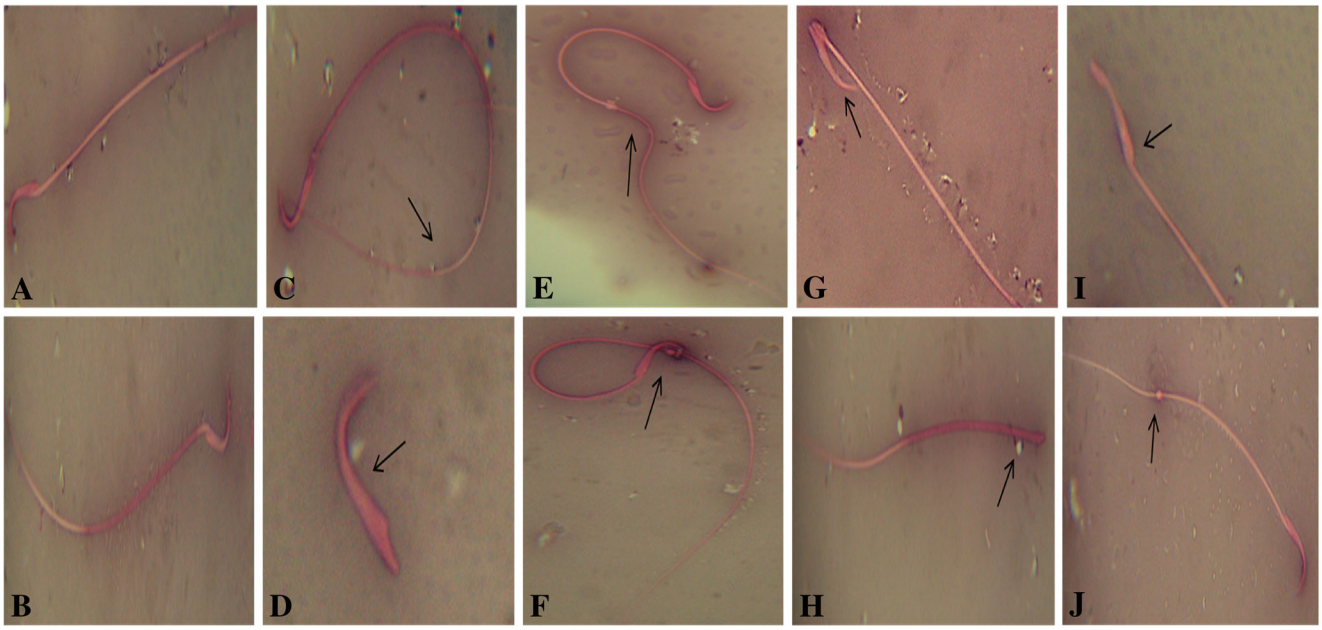

Fig. 2 Effect of $CdCl_2$ and SP and their co-exposure on sperm morphology of treated rats. *A* and *B* normal sperm, *C* looped tail, *D* detached tail, *E* curved tail, *F* coiled tail, *G* broken head, *H* detached head, *I* abnormal head shape, *J* cytoplasmic droplet

tubules. These tubules were almost devoid of spermatids and spermatozoa. Few leukocytes of neutrophils were infiltrated the tubular structures and the interstitium. Regarding to the interstitium, there was severe congestion of the interstitial blood vessels, edema, and hemorrhage (Fig. 4a 4). Cadmium and spirulina group (treatment) revealed moderate vacuolation in the testicular epithelium with slight improvement of spermatogenesis (Fig. 4a 5). Spirulina and cadmium group (prophylactic) showed normal seminiferous tubules with complete spermatogenesis. Sometimes, slight vacuolation in the spermatocytes was rarely seen (Fig. 4a 6).

Seminal vesicle

The seminal vesicle of control or SP-received rats showed normal epithelial lining and seminal fluid in its lumen (Fig. 4b 7). Cadmium-received group showed severe vacuolations (Fig. 4b 8), and hyperplasia in the lining epithelium (Fig. 4b 9) besides edema, congestion, hemorrhage, and few round cell infiltrations (Fig. 4b 10). Cadmium and spirulina group (treatment) revealed some nuclear stratification and scanty seminal fluid in the lumen (Fig. 4b 11). Meanwhile, the spirulina and cadmium group (prophylactic) showed normal fluid and lining epithelium (Fig. 4b 12).

Prostate glands

The prostate glands of control or spirulina-received rats revealed normal pattern and acini (Fig. 4c 13). Cadmium-received group showed severe dilation of some acini with presence of corpora amylacea (Fig. 4c 14), inflammatory edema with few round cell infiltrations (Fig. 4c 15), and hyperplasia and nuclear stratification in epithelial lining with no evidence of prostatic fluid (Fig. 4c 16). These epithelia were enlarged with vacuolated cytoplasm and large vesicular nuclei. Cadmium and SP group (treatment) revealed inactive lining epithelium and rare prostatic fluid (Fig. 4c 17). However, the SP and Cd group (prophylactic) showed normal prostatic fluid and hyperplasia in the lining epithelium (Fig. 4c 18).

Table 4 Effect of Cd and SP (treatment and prophylactic) serum hormonal level (testosterone, luteinizing, and estradiol hormones) of exposed rats (mean ± SE, $n=8$)

Items	Testosteron (ng/ml)	Luteinizing (mIU/ml)	Estradiol (pg/ml)
Control	35.16 ± 4.57^{b}	0.53 ± 0.01	24.12 ± 1.03^{a}
SP	76.55 ± 7.39^{a}	0.39 ± 0.29	19.74 ± 0.98^{a}
$CdCl_2$	15.34 ± 1.06^{c}	0.07 ± 0.01	18.39 ± 0.84^{a}
$CdCl_2$ + SP	33.96 ± 4.87^{b}	0.31 ± 0.24	19.20 ± 2.41^{a}
$CdCl_2$ then SP	24.58 ± 0.83^{b}	0.07 ± 0.01	9.63 ± 1.43^{b}

Means within the same column carrying different superscripts are significantly different ($p\leq0.05$)

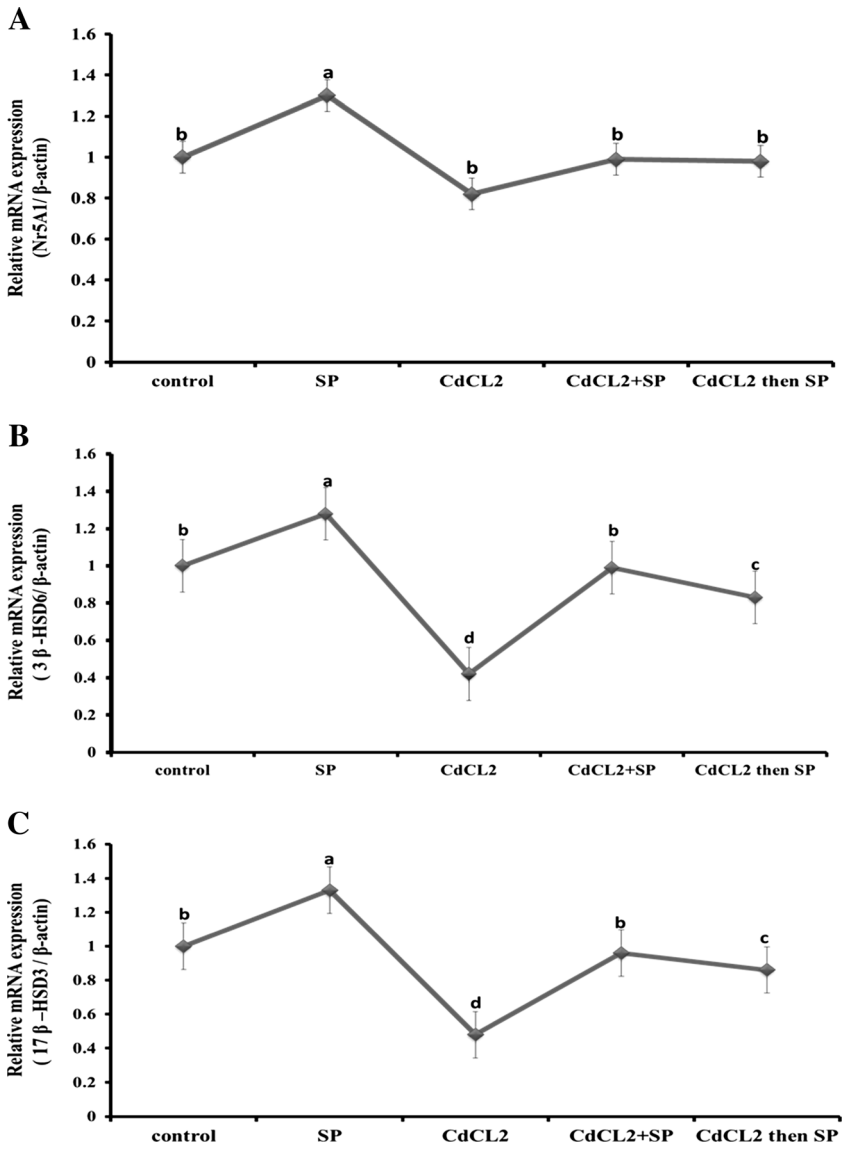

Fig. 3 Expressions of steroidogenic genes in rat testes. Real-time RT-PCR analysis of steroidogenic genes (Nr5A1, 3β-HSD6, and 17β-HSD3). β-actin was used as an internal control. Data expressed as mean ± SE (n= 3 replicates). *Columns carrying different superscripts* are significantly different (one-way ANOVA) ($p \leq 0.05$)

Discussion

This study was designed to investigate the beneficial effects of SP intake against the reproductive disorders induced by Cd in male rats. In the present work, it was found noticeable that subcutaneous administration of $CdCl_2$ in a daily dose of 2 mg/kg BW for 10 days to male rats revealed clear signs of toxicity such as a significant decrease in BW gain, testicular weight, sperm cell concentration, motility percent with concurrent increase in the percentage of sperm cell abnormalities accompanied with declined concentration of testosterone, and a significant downregulation of mRNA expressions of steroidogenic enzyme genes in testes of treated rats compared to control and SP co-administered groups.

Subcutaneous administration of cadmium chloride evoked several histopathological changes in the testes represented by testicular degeneration, desquamation, and necrosis in the germinal epithelium of the seminiferous tubules with the absence of spermatids and spermatozoa. Hyperplasia in the lining epithelium of seminal vesicle and prostate gland were also noticed indicating impaired spermatogenesis and steroidogenesis after Cd exposure.

The decreased BW following Cd intoxication is in agreement with the results of Gaurav et al. (2010) who reported that Cd reduced the BW, and this may be due to the ability of Cd in the induction of lipid peroxidation and consequently cell damage. Interestingly, the significantly decreased BW gain of Cd-administered rats was highly improved by prophylactic use of SP ($CdCl_2$ + SP) or administration of SP alone that could maintain the body gain of rats similar to control value. However, using of SP as treatment ($CdCl_2$ and then SP) could enhance the gain of animals. The ameliorative effects of SP on the BW gain could be also attributed to the supplementation of body by essential nutrients, like proteins, biochelating

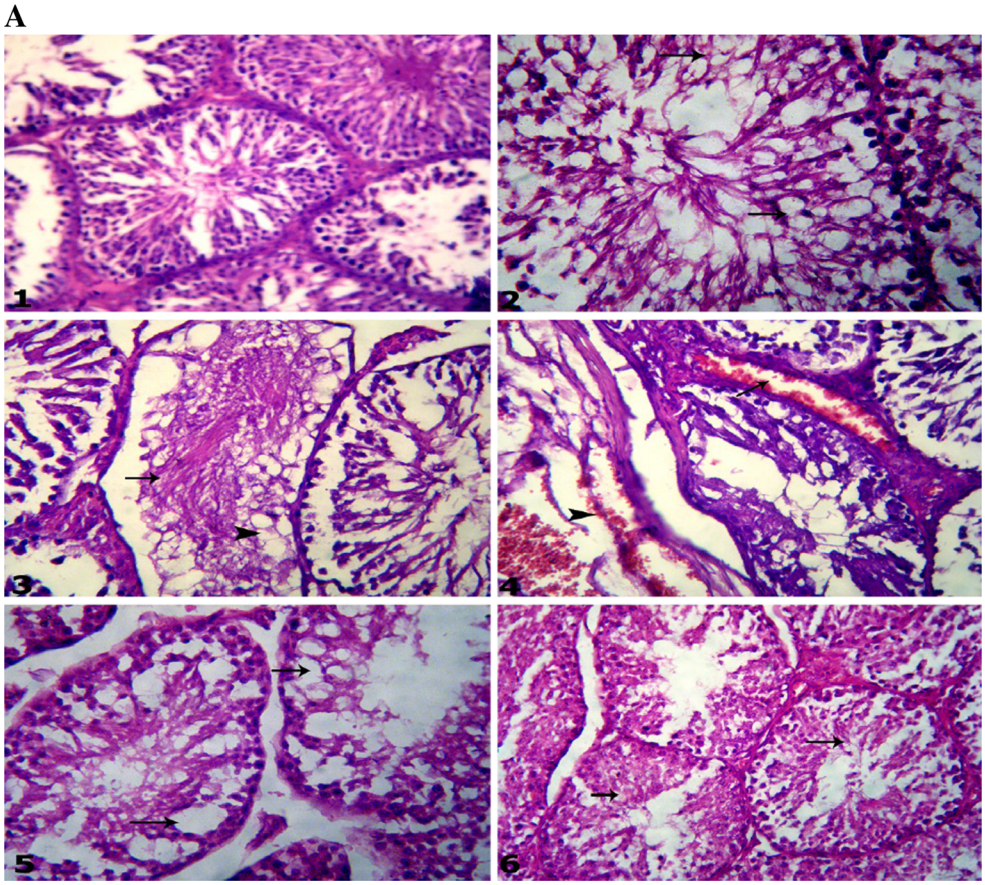

Fig. 4 **a** *1–6* Testis of rats from control group shows uniform seminiferous tubules with complete spermatogenesis and interstitial Leydig cells (*1*); cadmium-received group shows testicular degeneration represented by vacuolated germinal cells with rare spermatogenesis (*arrows*) (*2*) and necrosis in seminiferous tubule (*arrow*) (*3*) besides severe congestion (*arrow*) and hemorrhage (*arrowhead*) (*4*); cadmium and spirulina group (treatment) shows moderate vacuolation in the testicular epithelium (*arrows*) (*5*), and spirulina and cadmium group (prophylactic) shows normal seminiferous tubules with slight vacuolation and complete spermatogenesis (*arrows*) (*6*). HE ×400. **b** *7–12* Seminal vesicle of rats from control group shows normal epithelial lining and seminal fluid (*7*); cadmium-received group shows severe vacuolations (*arrows*) (*8*), and hyperplasia in the lining epithelium (*arrow*) (*9*) besides edema, congestion (*arrowhead*), hemorrhage, and few round cell infiltrations (*arrow*) (*10*); cadmium and spirulina group (treatment) shows nuclear stratification (*arrows*) and rare seminal fluid (*11*), and spirulina and cadmium group (prophylactic) shows normal fluid and lining epithelium (*arrows*) (*12*). HE ×400. **c** *13–18* Prostate gland of rats from control group shows normal pattern and acini (13× 100); cadmium-received group shows severe dilation of some acini (*arrowhead*) with presence of corpora amylacea (C), and hemorrhage (*arrow*) (14× 100), inflammatory edema with few round cell infiltration (*arrow*) (*15*) and hyperplasia and nuclear stratification in epithelial lining (*arrows*) with no evidence of prostatic fluid (*arrowhead*) (*16*); cadmium and spirulina group (treatment) shows inactive lining epithelium (*arrow*) and rare prostatic fluid (*arrowheads*) (*17*), and spirulina and cadmium group (prophylactic) shows normal prostatic fluid and hyperplasia in the lining epithelium (*arrows*) (*18*). HE ×400

vitamins, and amino acids (Mazo et al. 2004; Sharma et al. 2007). In addition, other constituents like β-carotene (Seshadri et al. 1991), SOD enzyme (Henrikson 1989), selenium, magnesium, zinc and manganese, α-lipoic acid, riboflavin, and some phytopigments such as xanthophyll, phycocyanin, and chlorophyll also have proven beneficial effects (Reddy et al. 2000; Careri et al. 2001; Chamorro et al. 2002).

Our results concerning the effect of $CdCl_2$ on testicular weight, structure, and functions as well as semen quality (sperm count, motility, and morphology) are in total agreement with those obtained by Wafaa et al. (2012) and Ekhoye et al. (2013). The decreased number of spermatozoa caused by $CdCl_2$ was also reported by Oteiza et al. (1999), Tbeileh et al. (2007), and Mudathir et al. (2008). Similarly, Hew et al. (1993) stated that $CdCl_2$ (1 mg/kg) as single I/P injection in rats resulted in the inability of sperms to release from epithelium of seminefrous tubules suggesting that Cd starts to exert its harmful effect on spermatogenesis in its early stage so decrease sperm production and sperm production efficiency.

Decreased sperm counts and relative testicular weight following Cd administration obtained in this study were associated with a significant decline in the serum testosterone level, and these findings run parallel with the results of Biswas et al. (2001), Santos et al. (2006), and Thompson and Bannigan (2008). Sperm production decrease in rat testes could be attributed to the decreased levels of testosterone which are very

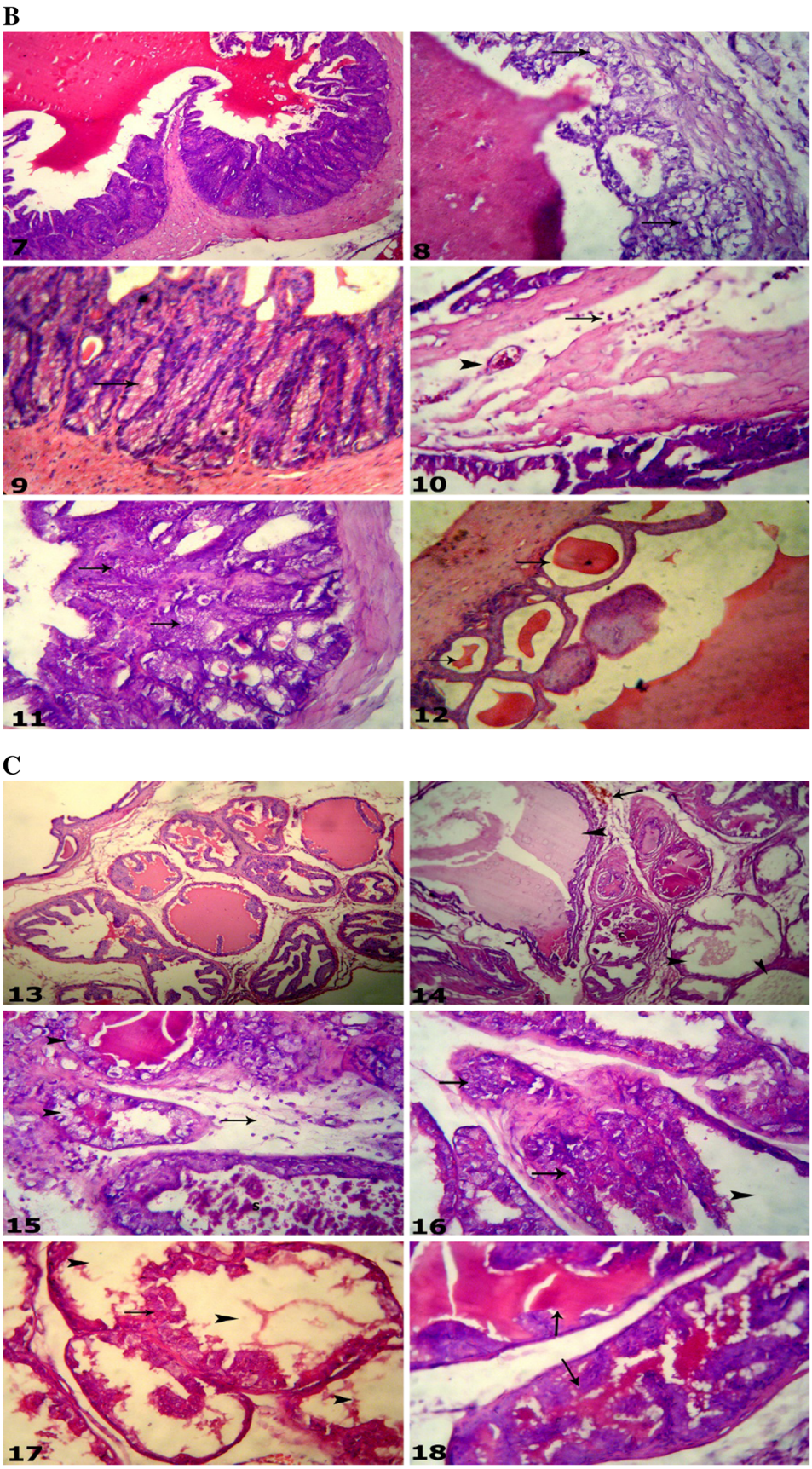

Fig. 4 (continued)

important to promote testicular growth. Besides, Cd exposure can interfere with hypothalamic-pituitary-testicular axis (Lafuente et al. 2000) and disrupt the initial steps of gamete production that are hormone-mediated and dependent (Gunnarsson et al. 2003).

The present results also ameliorated the testicular weight loss and the decreased sperm count after Cd treatment to the alterations in testicular morphology as represented by testicular degeneration, severe vacuolation, desquamation, and necrosis in the germinal epithelium of the seminiferous tubules which were almost devoid of spermatids and spermatozoa. These findings were also supported by Rekha et al. (2011), while Yang et al. (2006) reported return of the weight loss of testes to the adverse effects induced by Cd on germ cell count and the percent of elongated spermatids because the weight of testes depends largely on undifferentiated spermatogenic cell mass. In the same context, the testicular weight loss, disturbances in blood–testis barrier, necrotic changes, edema as well as reduction of germ cells have been found as results of cadmium-induced testicular injury (Siu et al. 2009).

Regarding the sperm motility, our data showed that Cd-administered rats exhibited a significant decrease in sperm motility. Similar result was obtained by Oliveira et al. (2006). The decreased sperm motility in Cd-treated rats could be attributed to the ability of Cd to bind with calmodulin instead of calcium inhibiting its role in sperm motility (Schlingmann et al. 2007). El-Missiry and Shalaby (2000) related the decrease in sperm concentration and motility % and the increase in the abnormal sperm following Cd administration to the ability of Cd to induce lipid peroxidation and necrotic and apoptotic changes in testicular tissue of rats due to changes in the levels of circulating androgen. $CdCl_2$ could induce testicular toxicity by a significant increase in hydroxyl free radical formation, generation of ROS, reduction of GSH content, and oxidative damage to macromolecules (Manna et al. 2008; Tremellen 2008; Liu et al. 2009). Peroxidative damage to the plasma membrane as a result of oxidative stress can interfere with functions of sperms as spermatozoa that contain high amounts of polyunsaturated fatty acids (PUFAs) increased its susceptibility to Cd-induced oxidative stress (Agrawal and Saleh 2002; Vernet et al. 2004). Another possible explanation for the impact of $CdCl_2$ on semen quality was that Cd could inhibit the activity of alkaline phosphatase enzyme (ALP) by competing with zinc that is essentially required for this enzyme and disrupt the zinc- and calcium-dependent cellular actions (Martin et al. 2007). These alterations observed in testicular structure and functions are highly correlated to the inhibition of spermatogenesis observed in this study which represented by downregulation of mRNA expression of enzyme genes (Nr5A1 mRNA, 3β-HSD6, and 17β-HSD3) besides defects in reproductive parameters and testicular degeneration.

With regards to relative testicular weight, co-administrated groups of SP either as prophylactic or as treatment could normalize the Cd-reducing effect on testicular weight. Moreover, administration of SP alone could numerically increase the motility of the sperms and significantly increase their counts than control group. In addition, the co-administration of SP with $CdCl_2$ (prophylactic) increased the motility % and sperm count to be comparable to those of control rats than $CdCl_2$ then SP (treatment) group. The prophylactic use of SP significantly decreased the % of sperm abnormalities than $CdCl_2$ group and gave results near to control values; the $CdCl_2$ then SP (treatment) group could also decrease the % of abnormal spermatozoa than $CdCl_2$ group but still significantly higher than control. This suggested the improving effects of SP on the quality of sperm parameters and fertility in male rats.

There was a significant decrease in serum level of TES in the $CdCl_2$ group compared to control. While, administration of SP alone showed a significant increase in serum TES level compared to control. On the other hand, $CdCl_2$ + SP group (prophylactic) and $CdCl_2$ then SP group showed non-significant differences in TES level than control group. The serum level of LH and EST hormone did not significantly differ among the all experimental groups. Administration of SP alone caused a prominent increase in testosterone concentration associated with an elevation in epididymal sperm counts and their motility indicating beneficial effects of SP intake on spermatogenesis. In addition, the testicular NR5A1, 3β-HSD6 mRNA, and 17β-HSD3 mRNA levels in the Cd + SP group were significantly higher than the Cd group. This suggests that SP intake can effectively recover Cd-induced deregulation of gene expression in early (NR5A1) and final (3β-HSD6 mRNA and 17β-HSD3) step in the steroidogenic pathway.

Concurrent treatment with SP significantly attenuated the alteration of reproductive system induced by $CdCl_2$ may be attributed to the presence of vitamins C and E; these antioxidant vitamins could protect the cells from the dangerous effect of free radicals as reported by Mathew et al. (1995) where vitamin E could inhibit the peroxidation of cell membrane lipids by trapping lipid peroxyl (LOO^{-}) and many other radicals to help in counteracting the oxidative damage and keeping the levels of GSH and ascorbic acid in damaged tissues caused by different xenobiotic as well as Cd toxicity (Rana et al. 1996; Patil and Rao 1999). Moreover, SP also contains selenium that is involved in the formation of selenium containing enzymes glutathione peroxidase and protein besides some other compounds like selenocystien, selenoglutathione, and selenodimethylselenide which are known to counteract the toxic effects of heavy metals (Simsek et al. 2009). In addition, to the presence of brilliant blue polypeptide phycocyanin and allophycocyanin which are important constituents of phycobilisomes (Bhat and Madyastha 2001; Riss et al. 2007), phycocyanin significantly inhibited

peroxyl radical-induced lipid peroxidation in rat liver microsomes (Bhat and Madyastha 2000). Phycocyanin compound of *Spirulina* may reduce the lipid peroxidation and has the ability to chelate metals including free iron (Premkumar et al. 2003). Chlorophyll and its derivatives in spirulina scavenge free radicals (Ferruzzi et al. 2002). Moreover, *Spirulina* was reported to enhance the activity of ALP enzyme (Sharma et al. 2007) and contain high amount of zinc (Jeyaprakash and Chinnaswamy 2005) which is required for this enzyme to enhance its role in improving semen quality.

In the present study, the S/C administration of Cd chloride to male albino rats evoked several histopathologic changes on testis, prostate gland, and seminal vesicle. The adverse effects of cadmium on histological structure of reproductive tissues have been reported even after administration of single doses where acute Cd injection caused some necrotic and apoptotic changes in the seminefrous tubular epithelium as well as edema, congestion, hemorrhage of testes besides diffuse necrosis of spermatozoa with impaired spermatogenesis as reported by Messaodi et al. (2010) and El-shahat et al. (2009). On the same context, Adamkovičová et al. (2010) also reported that I/P injection of a single dose of Cd (2 mg/kg body weight) to adult rats showed necrosis of seminiferous tubular epithelium, disruption of blood–testis barrier leading to edema and ischemia. Similar histological alterations were observed after administration of Cd in single dose in testes of mice (Massányi et al. 2008) and rabbits (Toman and Massányi 2002; Nemoto et al. 2009).

These effects of $CdCl_2$ on testicular tissue and fertility could be resulted as a consequence of induction of inflammatory reactions and increased expressions of TNF α and NO in testicular tissue (Gurl et al. 2007). Increased production of NO leads to more cellular damage as it could react with super oxide anion to generate peroxy nitrite radical and lead to depletion of intracellular GSH consequently increasing the susceptibility to oxidative disorders (Clancy and Abramson 1995), and this could explain the deficient spermatogenesis and testicular degeneration observed in the present study. Moreover, the alteration in testosterone levels induced by cadmium chloride may be the cause of the significant histopathological changes observed in testes. This result agrees with Tbeileh et al. (2007). Another mechanism explaining the testicular tissue damage caused by cadmium chloride is the decreased expression or lack of metallothionein (MT-1 and MT-2) genes after Cd administration (Waisberg et al. 2003).

Regarding the effect of oral administration of Cd on the histopathological findings in the prostate gland, the results showed severe dilation of some acini, presence of corpora amylacea, inflammatory edema, and hyperplasia of epithelial lining with no evidence of prostatic fluid. Similar results were reported by Alvarez et al. (2004) after oral Cd administration for 3 months suggesting the lack of the functionality of prostate gland and a decreased secretory capacity that accompanied with redox imbalance (Ramirez et al. 2002). Where, Cd has been reported to induce prostate TBARS as a result of lipid peroxidation that affect membrane integrity (Spatz 1992).

The pathological lesions induced by $CdCl_2$ in our study were remarkably reduced by the administration of SP as prophylactic and treatment suggesting that SP confers histological protection, and this came in harmony and support the earlier observation of the present investigation where administration of SP with $CdCl_2$ cause more significant improvement in the testicular structure and function. The role of SP in modulating the pathological alterations induced by metallic toxicity may be returned to the presence of β-carotene that is known to act as powerful antioxidant. The antioxidant mechanism of β-carotene has been suggested to be singlet oxygen quenching, free radical scavenging and chain breaking during lipid peroxidation (Krinsky and Deneke 1982; Gerster 1993). Luxia et al. (1996) reported that β-carotene of spirulina may decrease cell and macromolecule damage, particularly DNA, and help the repair and regeneration of damaged cells. Moreover, β-carotene reported to protect against oxidative damage of P450 systems in Leydig cells (Hanukoglu 2006).

Conclusion

From our findings, it is evident that administrating *S. platensis* alone could improve the parameters related to male fertility in rats. Moreover, concomitant exposure to *S. platensis* either as prophylactic or as treatment in Cd-intoxicated rats significantly ameliorated the Cd-induced alterations in the structure and functions of male reproductive system components and enhanced the semen quality parameters and fertility of rats. The role of *Spirulina* in reversing the toxic effects of cadmium may be due to the presence of several active constituents acting as free radical scavenging enzyme systems and provides protection against Cd-induced testicular damages.

Conflict of interest The authors declare that there are no conflicts of interest.

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