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RESEARCH ARTICLE

Antioxidant activity of *Spirulina platensis* alleviates doxorubicin-induced oxidative stress and reprotoxicity in male rats

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Abstract

Male infertility is a common side effect of doxorubicin (DOX) that substantially impairs the quality of life of young cancer survivors. Therefore, the current work was designed to evaluate the possible antioxidant and gonado-protective effects of *Spirulina platensis* (*S. platensis*) in DOX-treated rats. Intraperitoneal administration of DOX (3 mg/kg b.wt.) once weekly for 5 weeks significantly decreased the levels of testicular catalase, superoxide dismutase and glutathione peroxidase. Moreover, it significantly decreased serum testosterone, luteinizing hormone, and follicle-stimulating hormone levels, as well as sperm motility and sperm count. Additionally, DOX treatment significantly increased the testicular malondialdehyde concentration and the percent of sperm abnormalities and resulted in marked cystic dilation of seminiferous tubules with extensive separation, dissociation of germinal cells from the basement membrane and arrested spermatogenesis. Oral administration of *S. platensis* at a dose of 300 mg/kg daily for 5 weeks mitigated DOX-induced oxidative stress, testicular damage, hormone alterations and spermiogram abnormalities via its potent antioxidant activity. *S. platensis* may represent a potential therapeutic option to protect testicular tissue during DOX treatment.

Keywords Doxorubicin · *Spirulina platensis* · Oxidative stress · Fertility · Rats

Introduction

Doxorubicin (DOX), an anthracycline antibiotic, is a standout among the most widely used anticancer medications. It is used as an antitumor agent against human malignancies such as leukemia, lymphoma and many solid tumors (Lebrecht et al. 2007). DOX exerts its antitumor activities and adverse effects on other organs by the intracellular production of free radicals and reactive oxygen species (ROS) accompanied by DNA intercalation and consequent inhibition of topoisomerase II (Ichihara et al. 2007). Long-term treatment with DOX is limited mostly due to various toxicities, including cardiac (Abdel-Daim et al. 2017; Galal et al. 2013; Khafaga and El-Sayed 2018a), pulmonary (Mazzotta et al. 2016), hepatic (Injac et al. 2008), renal (Mohan et al. 2010; Yilmaz et al. 2006), hematological (Ahaus et al. 2000; O'Keefe and Schaeffer 1992) and testicular toxicity (Rizk et al. 2014).

Male infertility following DOX treatment is one of the obvious side effects and may be due to alterations in the spermiogram, as spermatogenic cells are one cell type that is susceptible to DOX-induced oxidative stress and apoptosis (Sikka 1996). Therefore, the concurrent administration of DOX with a potent antioxidant may be a suitable approach for reducing toxic side effects (Abushouk et al. 2017; Prahalathan et al. 2005).

Spirulina is a photosynthetic microalga. As it contains chlorophyll a, which is present in higher plants, botanists classify it as a microalga belonging to the Cyanophyceae class, but bacteriologists classify it as a bacterium because of its prokaryotic structure. Spirulina belongs to the *Oscillatoriaceae* family, which acquired the ability for photosynthesis before any other organism and is considered the precursor from which higher plants evolved (Desai and Sivakami 2004).

Apart from the high (up to 70%) protein content, Spirulina also contains vitamins such as B_{12} and pro-vitamin A (β-carotenes) and minerals such as iron. It is also rich in phenolic acids, tocopherols and γ-linolenic acid. In addition

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to basic nutrients such as amino acids, essential fatty acids, vitamins and minerals, Spirulina provides many phytonutrients that are lacking in most human diets (Dillon et al. 1995).

Spirulina contains potent naturally occurring antioxidants and free radical scavengers (Aissaoui et al. 2017; El-Tantawy 2016). Furthermore, Spirulina has anti-inflammatory, immune-modulatory, hepato-protective (Abdel-Daim et al. 2015; Khafaga and El-Sayed 2018b), neuro-protective (Chattopadhyaya et al. 2015; Perez-Juarez et al. 2016; Thaakur and Sravanthi 1996), cardio-protective (Ibrahim and Abdel-Daim 2015; Khan et al. 2005a), nephro-protective (Abdel-Daim et al. 2013, 2016; Saber et al. 2015), gonado-protective (Bashandy et al. 2016; Yener et al. 2013) and anticancer activities (Basha et al. 2008; Flores Hernandez et al. 2017; Konickova et al. 2014). It is considered as an excellent food with anti-viral, and a strong antioxidant properties (Kulshreshtha et al. 2008; Lee et al. 1998).

Interest in Spirulina is based on the fact that it is non-toxic and bioavailable and provides significant multi-organ protection against numerous drugs and chemical-induced toxicity (Khan et al. 2005b). Spirulina has been reported to improve organ toxicities induced by chemotherapeutic agents such as cisplatin, DOX, and cyclosporine (Mohan et al. 2006). Therefore, the present study aimed to evaluate the possible antioxidant and gonado-protective effects of *Spirulina platensis* (*S. platensis*) in DOX-treated rats.

Materials and methods

Spirulina tablets were purchased from DXN Marketing Company (Malaysia) as green tablets, with each tablet containing 250 mg of *S. platensis*. DOX (adriamycin®) is a product of Pfizer that is available as an injectable solution (10 mg doxorubicin per 5 ml).

Experimental animals

The current study involved 40 adult male albino rats weighing 150–180 g obtained from the laboratory animal house at the Faculty of Veterinary Medicine, Zagazig University. The rats were housed in metal cages at 23 ± 2 °C and 40–60% relative humidity with a 12 h light cycle. Throughout the experimental period, rats were fed a rodent diet, and water was provided *ad libitum*. Rats were adapted to the experimental location for 2 weeks. Animal housing and management and the experimental protocols were conducted as stipulated in the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health (NIH) and as approved by the local authorities of Zagazig University, Zagazig, Egypt.

Experimental protocol

After 2 weeks of acclimatization to the laboratory environment, 40 adult male rats were randomly allocated into four equal groups as follows: Group I, Control group: each rat was gavaged with 0.5 ml of normal saline once daily for 5 weeks; Group II, Spirulina-treated group (SP): each rat was gavaged with *S. platensis* (300 mg/kg b.wt.) (Simsek et al. 2009) dissolved in normal saline once daily for 5 weeks; Group III, DOX-treated group (DOX): each rat received DOX (3 mg/kg b.wt.) intraperitoneally once weekly for 5 weeks (Patil and Balaraman 2009); and Group VI, SP+DOX: each rat received *S. platensis* before DOX and concurrently with doxorubicin at the previously mentioned doses and duration.

Sampling

At the end of the experimental period (5 weeks), rats were sacrificed under light anesthesia. Five blood samples from each group were collected in test tubes without EDTA, left for 20 min at room temperature to clot, and centrifuged at 3000 rpm for 20 min to obtain serum, which was stored at − 80 °C until the hormonal analysis. For sperm collection, the cauda epididymis of one testis of each rat was removed and transferred to a sterilized Petri dish containing 2 ml of warm (37 °C) normal saline; then, a small opening was made using sterilized scissors to facilitate the passage of sperm from the epididymis to obtain a suspension of the epididymal content for spermiogram analysis. Immediately after each rat was killed, both testes were removed and washed in physiological saline. One testis from each rat was preserved at − 80 °C for the subsequent preparation of tissue homogenate to evaluate testicular oxidative/antioxidant indices, and the other testis was preserved in neutral buffered formalin (10%) for histopathological examination.

Testicular oxidative/antioxidant status

Testes were rapidly thawed, and half a gram of tissue from one testis of each rat was electrically homogenized in 5 ml of cold phosphate buffer (pH 7.4) and then centrifuged at 3000 rpm for 20 min. The resulting supernatants were removed and used to measure the levels of glutathione peroxidase (GPx) according to the methods detailed by Beutler et al. (1963), superoxide dismutase (SOD) according to the method reported by Nishikimi et al. (1972), catalase (CAT) according to the methods of Aebi (1984) and malondialdehyde (MDA) concentration according to Mihara and Uchiyama (1978).

Hormone analysis

Serum testosterone was measured using a rat ELISA kit provided by Cusabio (Wuhan, China). Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels were determined using rat ELISA kits from My BioSource (San Diego, CA, USA) according to the manufacturer's protocol. *Briefly,* ELISA kit applies the quantitative sandwich enzyme immunoassay technique. The microtiter plate has been pre-coated with monoclonal antibody specific for the tested hormone. Standards or samples are added to the appropriate microtiter plate wells with an antibody specific and Horseradish Peroxidase (HRP)-conjugated polyclonal antibody, specific for the tested hormone and incubated. Next, substrate solutions are added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period. The enzyme-substrate reaction is terminated by addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm within 10 min. A standard curve is plotted relating the intensity of the color to the concentration of standards. The tested hormone concentration in each sample is interpolated from this standard curve.

Sperm analysis

Sperm motility was determined according to (Galal et al. 2016; Slott et al. 1991). Sperm count was measured according to the method reported by Robb et al. (1978), and abnormalities were evaluated as previously described by Narayana et al. (2005).

Histopathological examination

The testes preserved in 10% neutral buffered formalin were dehydrated in a graded ethanol series (70–100%), cleared in xylene, and embedded in paraffin. Sections (5 µm thickness) were prepared, stained with hematoxylin and eosin and then examined microscopically (Suvarna et al. 2013).

Statistical analysis

The data are presented as the mean ± SE for each group. The variation between groups was statistically analyzed using one-way analysis of variance (ANOVA) followed by Duncan's multiple ranges Post hoc test for pairwise comparisons. Differences were considered significant at $P < 0.05$.

Results

Effect of *S. platensis* and DOX on testicular oxidative/antioxidant status

Daily oral administration for 5 weeks of 300 mg *S. platensis*/kg b.wt. to adult male rats significantly ($P < 0.05$) increased the levels of testicular SOD, CAT, and GPx and significantly decreased the MDA concentration compared with control treatment. While, intraperitoneal administration of DOX alone once weekly for 5 weeks at a dose of 3 mg/kg b.wt. caused a significant ($P < 0.05$) decrease in the levels of testicular SOD, CAT, and GPx, the MDA concentration showed a different pattern. Interestingly, the antioxidant status of testicular tissue from DOX-treated rats was improved by pretreatment and concurrent treatment with *S. platensis* (Fig. 1).

Effect of *S. platensis* and DOX on some reproductive hormones

Serum testosterone, LH and FSH levels in rats significantly ($P < 0.05$) decreased in response to intraperitoneal injection of DOX compared with control. Meanwhile, co-treatment with *S. platensis* and DOX significantly ameliorated serum testosterone, LH and FSH levels compared with the DOX group (Fig. 2).

Effect of *S. platensis* and DOX on spermiogram

Oral administration of *S. platensis* to adult male rats daily for 5 weeks resulted in a significant ($P < 0.05$) decrease in the percent of sperm abnormalities and an increase in sperm count compared with control treatment. Intraperitoneal DOX administration once weekly for 5 weeks caused a significant increase in the percent of sperm abnormalities and decreased the percent motility and sperm count. Luckily, co-treatment with *S. platensis* and DOX greatly improved semen parameters compared with DOX alone (Fig. 3).

Histopathological findings

Testicular tissue sections from rats treated with *S. platensis* showed normal histological architecture (Fig. 4a, b), whereas those from the DOX-treated group showed marked cystic dilation of seminiferous tubules with extensive separation, dissociation of germinal cells from the basement membrane (Fig. 4c), and arrested spermatogenesis (Fig. 4d). Moreover, severe interstitial edema (Fig. 4e) and severe congestion of blood vessels in interstitial tissue (Fig. 4f) were observed. However, the examined testes sections from rats co-treated with *S. platensis* and DOX revealed slight interstitial edema and slight dissociation of spermatogonial cells (Fig. 4g), with an appropriate percentage of spermatozoa in seminiferous tubules and Leydig cells with a normal appearance (Fig. 4h).

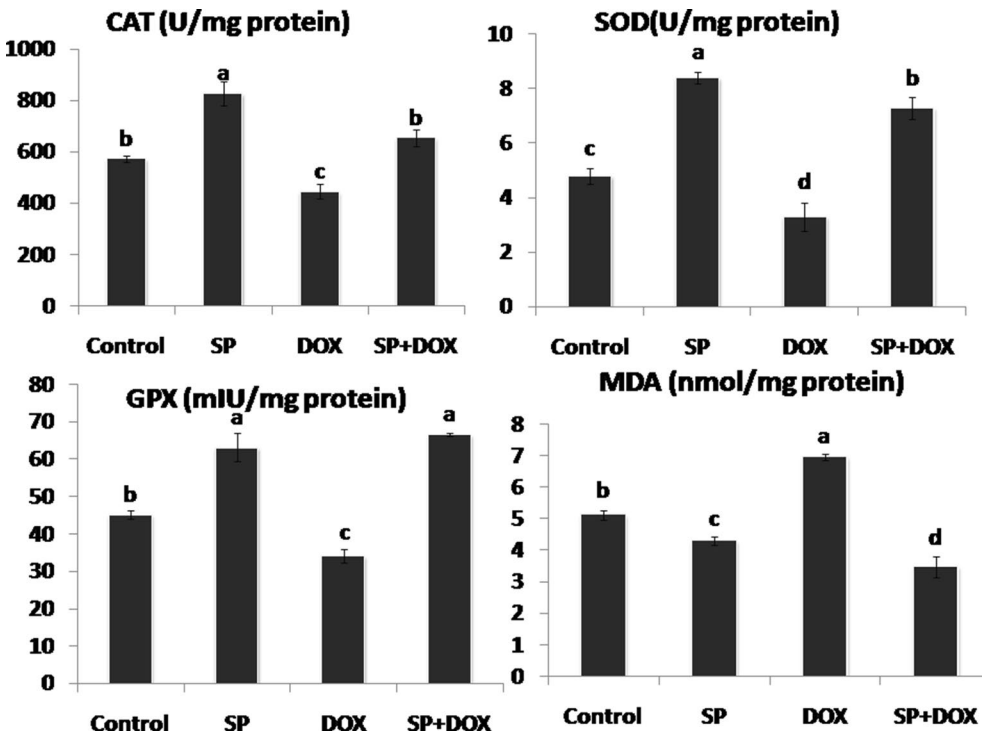

Fig. 1 Effect of daily oral administration of *S. platensis* at a dose of 300 mg/kg b.wt. for 5 weeks on testicular CAT, SOD, GPx and MDA in doxorubicin-treated rats. Data are presented as the mean ± SE. Bars with different superscripts are significantly different (one-way ANOVA followed by Duncan's multiple range test, $P < 0.05$, n = 5/group)

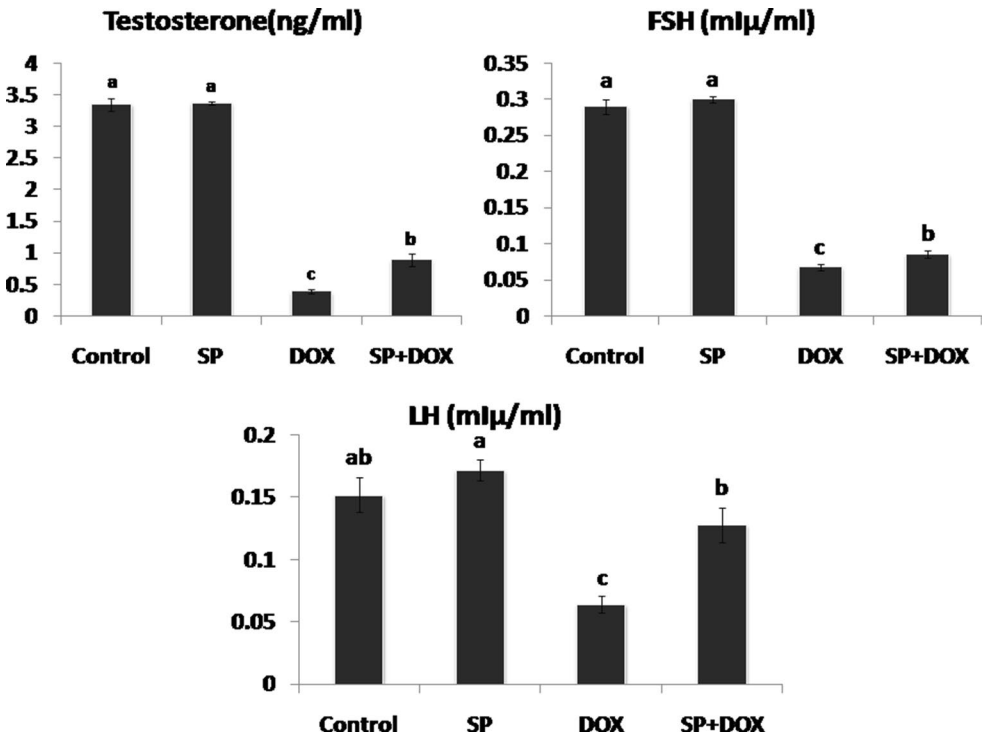

Fig. 2 Effect of daily oral administration of *S. platensis* at a dose of 300 mg/kg b.wt. for 5 weeks on serum testosterone, FSH and LH in doxorubicin-treated rats. Data are presented as the mean ± SE. Bars with different superscripts are significantly different (one-way ANOVA followed by Duncan's multiple range test, $P < 0.05$, n = 5/group)

Discussion

Daily oral administration of *S. platensis* to adult male rats at a dose (300 mg/kg b.wt.) for 5 weeks induced a significant increase in testicular CAT, SOD, and GPx levels and a significant decrease in MDA concentration compared with control treatment. This improvement in the testicular antioxidant status of *S. platensis*—treated

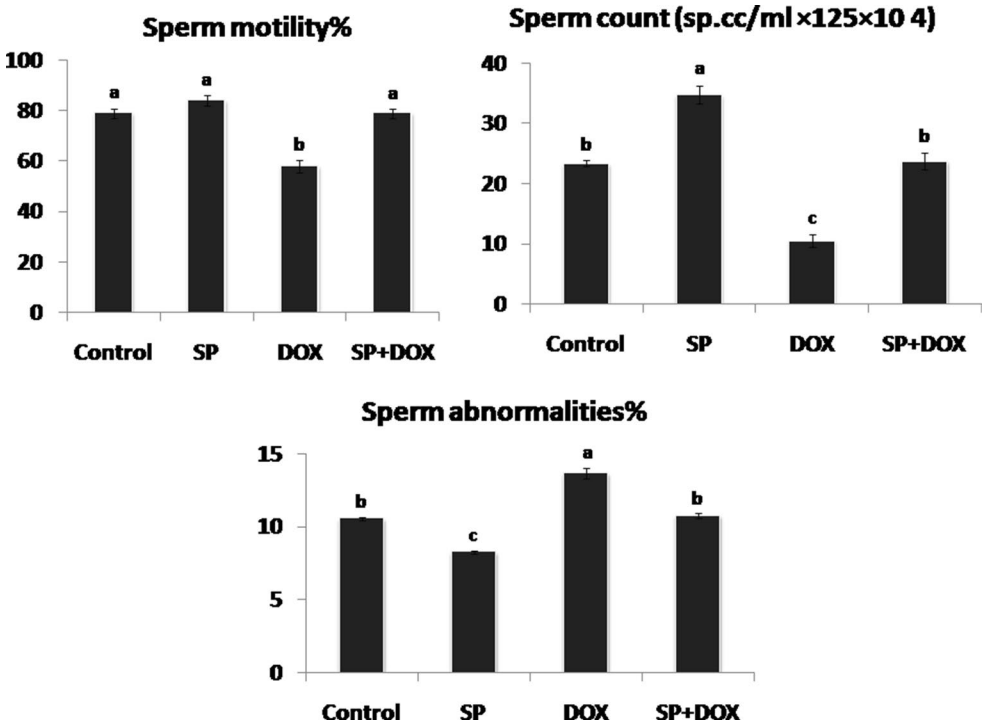

Fig. 3 Effect of daily oral administration of *S. platensis* at a dose of 300 mg/kg b.wt. for 5 weeks on percent sperm motility, sperm count and percent sperm abnormalities in doxorubicin-treated rats. Data are presented as the mean ± SE. Bars with different superscripts are significantly different (one-way ANOVA followed by Duncan's multiple range test, $P < 0.05$, n = 5/group)

rats may be due to the high content of active antioxidant constituents in Spirulina, such as C-phycocyanins, β-carotene, minerals, and vitamins. The anti-oxidative effects of Spirulina could be explained by direct inhibition of lipid peroxidation and free radical scavenging or indirect enhancement of SOD and CAT activity (Aissaoui et al. 2017; Upasani and Balaraman 2003).

The present study indicated that intraperitoneal DOX administration at a dose of 3 mg/kg b.wt. once weekly for 5 weeks induced testicular oxidative stress that was evidenced by reduced testicular CAT, SOD, and GPx levels and an elevated MDA concentration. These findings may be attributed to the generation of ROS, which exhaust CAT, SOD and GPx, ultimately leading to oxidative damage to the cell membrane, which was indicated by the increased MDA concentration (Mimnaugh et al. 1985). Our results are supported by a previous study of Hozayen et al. (2013) that showed that intraperitoneal injection of 4 mg DOX/kg b.wt. for 6 weeks induced a marked elevation in testicular lipid peroxidation and a significant decrease in the levels of glutathione, CAT, SOD and peroxidase in male albino rats.

The improvement in the testicular antioxidant status of DOX-treated rats by *S. platensis* administration was evidenced by significant increases in CAT, SOD and GPx activity and the reduction in MDA concentration compared with the DOX group; this finding may be attributed to the potent antioxidant components of Spirulina, such as carotene, vitamin C, vitamin E, selenium, manganese and C-phycocyanin, that prevent cellular damage caused by oxidative stress in testicular tissue (Mazo et al. 2004). Daily oral administration of *S. platensis* protects against DOX toxicity via attenuation of lipid peroxidation and decreased production of free-radical derivatives (Abdel-Daim 2014).The results of the current study are strengthened by those of Bashandy et al. (2016), who found that concurrent administration of arsenic and *S. platensis* at a dose of 300 mg/kg b.wt. resulted in elevated testicular SOD, CAT and GSH levels and a reduced MDA concentration compared with arsenic monotherapy.

Intraperitoneal administration of DOX once weekly for 5 weeks to adult male rats caused a marked decrease in serum testosterone, LH, and FSH levels compared with control treatment. These results clarified that DOX alters the function of the anterior pituitary (LH and FSH production) and Leydig cells (testosterone production) via induction of oxidative stress. The reduced LH and FSH levels might be attributed to disturbance of the negative feedback control of the hypothalamic-pituitary axis (Kovacs et al. 1997). Additionally, pituitary dysfunction in terms of LH release may result from damage to the cell membrane-mediated signaling mechanisms involved in releasing LH (Atessahin et al. 2006). Steroidogenesis in male rats is stimulated by hypothalamic gonadotropin releasing hormone (GnRH), which stimulates the production and release of pituitary LH, which binds to the LH receptor (LHR) on the surface of Leydig cells to up-regulate testosterone production; thus, the reduction in testosterone levels is a logical consequence of the reduction in LH levels (McVey et al. 2008). Moreover, antineoplastic agents can directly disrupt Leydig cells; therefore,

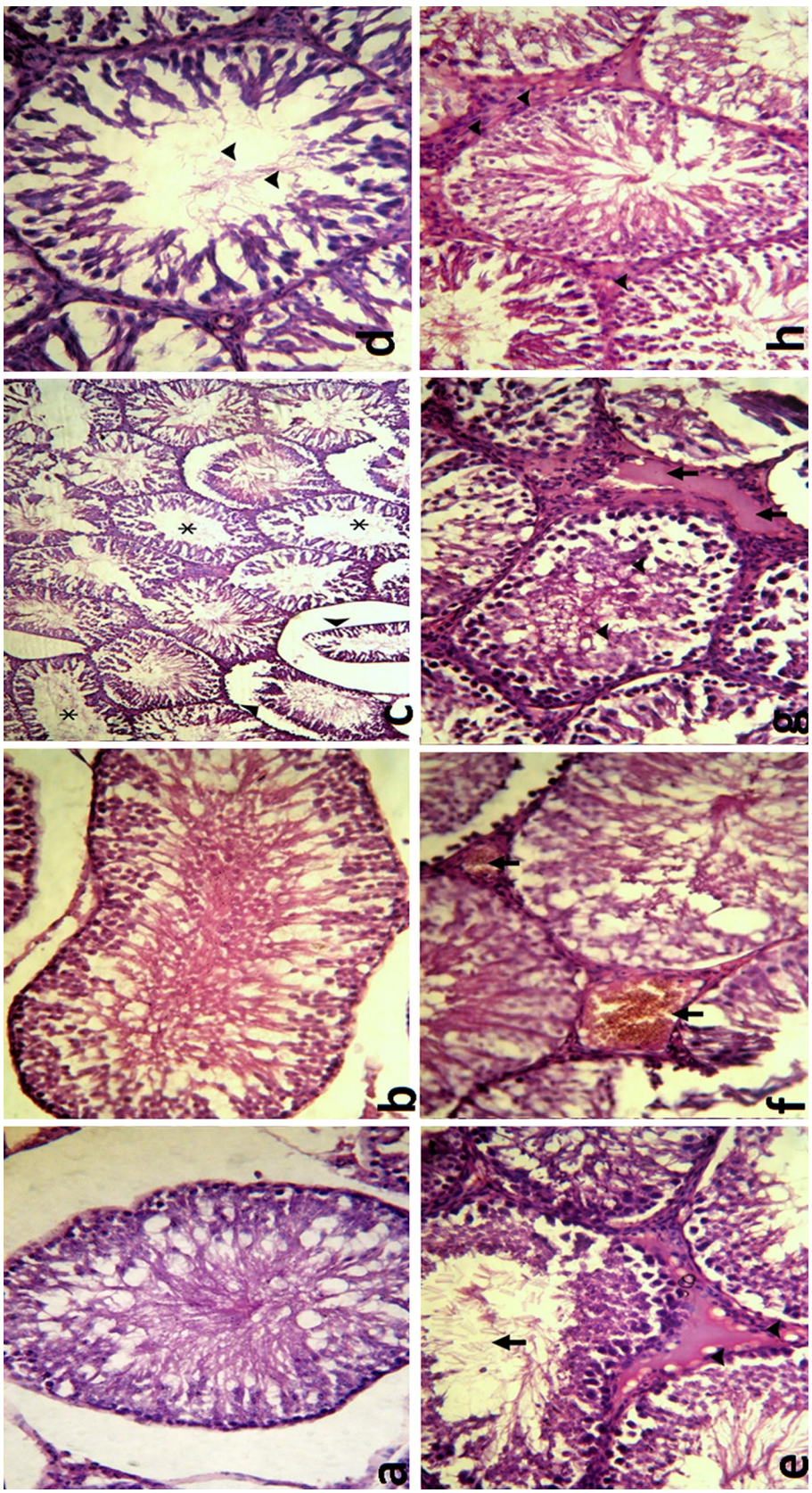

Fig. 4 Testicular tissue sections from the control group (**a**) and the Spirulina-treated group (**b**) showing normal histological structures of seminiferous tubules with normal spermatogenesis and an appropriate percentage of spermatozoa (H&E, ×400). Testicular tissue section from the DOX-treated group showing marked cystic dilation of seminiferous tubules (asterisks) with separation of germinal cells from the basement membrane (arrowheads) (H&E, ×100) (**c**); cystic dilatation of seminiferous tubules with arrested spermatogenesis (arrowheads) and dissociation of spermatogonial cells (H&E, ×400) (**d**); severe interstitial edema (arrowheads) with cystic dilatation of seminiferous tubules (arrow), arrested spermatogenesis and dissociation of spermatogonial cells (H&E, ×400) (**e**); and severely congested blood vessels in interstitial tissue (arrows) (H&E, ×400) (**f**). Testicular tissue section from the SP + DOX group showing slight interstitial edema (arrows) with an appropriate percentage of spermatozoa in seminiferous tubules (arrowheads) and slight dissociation of spermatogonial cells (H&E, ×400) (**g**) and an appropriate percentage of spermatozoa in seminiferous tubules, normal Leydig cells appearance (arrowheads) and slight dissociation of spermatogonial cells (H&E ×400) (**h**)

the reduction in circulating testosterone is hypothesized to stem from the direct toxic effect of DOX on Leydig cells (Badkoobeh et al. 2013). Similarly, Rizk et al. (2014) found that intraperitoneal DOX treatment of adult rats with a total cumulative dose of 18 mg/kg b.wt. for 3 weeks significantly decreased serum testosterone, FSH and LH levels by 83.37, 52.38, and 45.56%, respectively, compared with control treatment.

Simultaneous administration of *S. platensis* and DOX to adult male rats improved pituitary and Leydig cell function, which manifested as significant increases in serum testosterone, LH and FSH levels compared with the DOX-treated group. These results may be due to the presence of many endogenous antioxidants in *S. platensis*, which reduce oxidative stress and ameliorate the pathological changes induced by DOX in the testis (Bashandy et al. 2016). Along the same line, Farag et al. (2016) reported that Spirulina administration at a dose of 150 mg/kg b.wt. daily for 10 days to cadmium-intoxicated rats significantly increased the testosterone level compared with cadmium treatment alone.

Daily oral administration of *S. platensis* to adult male rats for 5 weeks caused a significant increase in sperm count and a significant decrease in sperm abnormalities compared with control treatment. This improvement in the spermiogram analysis was confirmed by the normal histopathological structure of testicular tissue and was potentially due to the presence of potent natural antioxidants and free radical scavengers in Spirulina that protect testicular tissue from damage (Basha et al. 2008). Our results are supported by those obtained by Farag et al. (2016), who reported that *S. platensis* treatment alone could improve parameters related to male fertility in rats, as evidenced by increasing sperm motility and sperm count.

In fact, male germ cells are one tissue that is susceptible to various environmental substances and drugs (Abu Zeid et al. 2016; Galal et al. 2016), such as DOX (Hou et al. 2005). In the current study, DOX treatment resulted in the deterioration of spermatogenesis, which was represented by reductions in sperm count and percent motility, as well as a significant increase in the percent of sperm abnormalities. In addition, the histopathological analysis of testicular tissue from the DOX group revealed deterioration of testicular tissue and arrested spermatogenesis. Likewise, Badkoobeh et al. (2013) noted that the epididymal sperm count decreased after DOX treatment, while the number of dead and abnormal sperm increased in adult male Wistar rats.

DOX-induced cytotoxicity is mainly concentrated in early spermatogenic cells, which undergo rapid proliferation and differentiation (Choudhury et al. 2000). The high rates of DNA synthesis and cellular proliferation in these cells may play a role in their susceptibility to damage by free radicals (Sikka 2004). DOX cytotoxicity is responsible for producing apoptotic round spermatids and multinucleated apoptotic cells. The intercalation of doxorubicin in germ cell DNA during division is considered the principal reason for cellular death in the seminiferous epithelium (Konopa 1988; Shinoda et al. 1999).

Oral administration of *S. platensis* to DOX-treated rats resulted in a significant improvement in sperm parameters compared with doxorubicin monotherapy. These improvements may be due to the better testicular antioxidant status and the increased levels of testosterone, LH and FSH, which led to improved spermatogenesis. These results were confirmed by the increased sperm count evidenced by histopathological examination of testicular tissue from this group. Our results are supported by those of Farag et al. (2016), who found that administration of *S. platensis* either as a prophylactic or treatment significantly reduced sperm abnormalities compared with control in cadmium-intoxicated rats. Similarly, Bashandy et al. (2016) reported that Spirulina administration at a dose of 300 mg/kg b.wt. attenuated the oxidative stress, testicular damage, and sperm abnormalities in arsenic-intoxicated rats.

Conclusion

The results of the current study strengthen the vital role of oxidative injury in reprotoxicity resulting from DOX treatment. Furthermore, the prevention of oxidative stress by the natural antioxidant *S. platensis* could be considered a promising strategy for ameliorating DOX-induced reprotoxicity in rats.

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Compliance with ethical standards

Ethical statement Animal housing and management and the experimental protocols were conducted as stipulated in the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health (NIH) and were approved by the local authorities of Zagazig University, Zagazig, Egypt.

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

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