Chlorella Vulgaris and Spirulina Platensis Mitigate Lead Acetate-induced Testicular Oxidative Stress and Apoptosis with Regard to Androgen Receptor Expression in Rats

Ibrahim Ibrahim
Suez Canal University Faculty of Veterinary Medicine
Abeir Shalaby
Suez Canal University Faculty of Veterinary Medicine
Raghda Abd Elaziz
Suez Canal University Faculty of Veterinary Medicine
hoda bahr (Hoda_hassona@vet.suez.edu.eg)
Suez Canal University Faculty of Veterinary Medicine https://orcid.org/0000-0002-7012-8427

Research Article

Keywords: Lead. C. vulgaris. S. platensis. oxidative stress. Caspase-3. androgen receptor

Posted Date: February 12th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-182452/v1

Version of Record: A version of this preprint was published at Environmental Science and Pollution Research on March 22nd, 2021. See the published version at https://doi.org/10.1007/s11356-021-13411-w.

Abstract

The current research was constructed to throw the light on the protective possibility of *Chlorella vulgaris (C. vulgaris)* and *Spirulina platensis* (*S. platensis*) versus lead acetate- prompted testicular dysfunction in male rats. Forty rats were classified into four groups; i) control, ii) rats received Lead acetate (30 mg/kg bw), iii) rats were concomitantly received Lead acetate and *C. vulgaris* (300 mg/Kg bw), vi) rats were simultaneously treated with Lead acetate and *S. platensis* (300 mg/Kg bw) via oral gavage for 8 weeks. Lead acetate promoted testicular injury as expressed with fall in reproductive organ weights, and gonadosomatic index (GSI). Spermatogenesis disruption is indicated by Sperm cell count reduction, and increased sperm malformation percentage. Steroidogenesis deterioration is evoked by minimized serum testosterone along with maximized follicle-stimulating hormone (FSH), luteinizing hormone (LH) levels. Testicular oxidative, inflammatory, and apoptotic cascades are revealed by elevated Acid phosphatase (ACP) and Sorbitol Dehydrogenase (SDH) serum leakage, declined testicular total antioxidative capacity (TAC) with elevated total oxidative capacity (TOC), tumor necrosis factor alpha (TNF-α), Caspase-3 levels, lessened androgen receptor (AR) expression, and histopathological lesions versus control. Our research highlights that *C. vulgaris* or *S. platensis* therapy can modulate lead acetate-promoted testicular dysfunction via antioxidant, immunomodulatory, anti-apoptotic potentials promoted testicular histoarchitecture, and androgen receptor restoration with better impacts to *S. platensis* comparing to *C. vulgaris*.

Introduction

Male fertility has been minified worldwide that may contribute to environmental pollutants (Sukhn et al. 2018).

Lead (Pb) is one of heavy metal and possess non-biodegradable nature favoring its environmental accumulation with increasing hazards (Goto et al. 2020). Pb exposure is represented in food, air, water, paint, ceramics, cosmetics, and leaded gasoline as well as occupational sources (Adela et al. 2012; Ali et al. 2020).

Previous researches reported Pb-prompted reproductive system oxidative burden along with subsequently disrupted steroidogenesis and spermatogenesis in humans and animals (Haw et al. 2012; Udefa et al. 2020).

Androgen receptor (AR) has fundamental roles in spermatogenesis and male fertility. Testosterone acts directly on both Sertoli cells, spermatogenic cells to enhances spermatogenesis via AR that requiring for normal spermatids differentiation and liberation from seminiferous epithelium (Hazra et al. 2013). Moreover, Testosterone supports Leydig cells development and functions via AR and motivates growth and function of male reproductive system. Luteinizing Hormone (LH) motivates steroidogenesis via LH receptor on the Leydig cells (Wang et al. 2009; Wang et al. 2018). However, Follicle Stimulating Hormones

(FSH) needed for normal functions and number of Sertoli cells that support germ cells mitotic activity through spermatogenesis stages (Oduwole et al. 2018).

In this regard, (Mokhtari and Zanboori 2011) validated that daily oral treating with lead acetate solution (50 and 100 mg/kg) for 28 days minimized sera testosterone and affected on sexual behavior, while the lower dose (25 mg/kg) didn't exert any significance difference. Further, orally administration of lead acetate (60 mg/kg) for 28 days decreased weight of testis, sperm count, motility and viability percentage (Offor et al. 2017).

Many researches provoked the nutritional and therapeutic importance of microalgae (Camacho et al. 2019; Sajilata et al. 2008). *Chlorella vulgaris* and *Spirulina platensis* safety is well pronounced by the FDA comparing to other algae species (Bauer et al. 2017).

Chlorella vulgaris (*C. vulgaris*) is a microalgae distributed in freshwater and rich in antioxidants, chlorophyll pigments, polysaccharides, amino acids, calcium, phosphorus, iron, iodine, manganese, omega-3, omega-6 polyunsaturated fatty acids, carotene, vitamin C and E (Rahimnejad et al. 2017). Importantly, *C. vulgaris* reversed cancer in human lung cancer H1299 (Wang et al. 2010), mercury-induced renal toxicity (Blas-Valdivia et al. 2010), expression of brain c-fos in forced swimming stress (Souza Queiroz et al. 2016), diazinon- caused hepatic, splenic oxidative and inflammatory burden (Abdelhamid et al. 2020).

Spirulina platensis (*S. platensis*) is a filamentous planktonic algae and possess great nutritional value such as protein, fatty acids, vitamins, iron, polysaccharides, carotenoids, chlorophyll, and C-Phycocyanin pigment (Hosseini et al. 2013). Due to these active ingredients, many studies highlighted that *S. platensis* has antioxidant (Barkallah et al. 2020), immunomodulator (Wu et al. 2016), hypolipidemic (Kata et al. 2018), antidiabetic (Gargouri et al. 2016), and anticancer (Czerwonka et al. 2018) possibilities. Additionally, *S. platensis* pronounced immunomodulatory, and anti-inflammatory possibilities in Egyptian Baladi bucks versus erythromycin (Abdel Daim 2014), in rat colitis model (Abdel-Daim et al. 2015), in mice heart versus Tilmicosin (Ibrahim and Abdel-Daim 2015), and in lead acetate intoxicated rabbits (Aladaileh et al. 2020). Importantly, *S. platensis* counteracted reprotoxicity promoted by Sodium Arsenite in rats (Bashandy et al. 2016). Furthermore, *S. platensis* mitigated deltamethrin (Abdelkhalek et al. 2015), and aflatoxin B1 (Abdel-Daim et al. 2020) intoxication in Nile tilapia.

There is no previously published data demonstrating the impact of *C. vulgaris* and *S. platensis* on androgen receptor expression in lead acetate treated rats. Hence, the current research was constructed to throw the light on the effect of *C. vulgaris* and *S. platensis* versus lead acetate- prompted testicular dysfunction in male rats via analysis of sera testicular enzymes activities, hormones profile, testicular oxidant/antioxidant status, tumor necrosis factor alpha (TNF-α), casapase-3, and androgen receptor expressions.

Materials And Methods

Laboratory Animals

This research was carried using forty male Wistar rats with 130-180 g as initial body weight. Rats were acclimatized for two weeks with food and drinking water supplied ad libitum. Study protocol was complied with the ethical guidelines for laboratory animals use at the Faculty of Veterinary Medicine, Suez Canal University, Egypt (approval NO.).

Materials list

Lead acetate trihydrate was obtained from Oxford Lab. Co., India (Catalog# 400002). *C. vulgaris* and *S. platensis* -lyophilized powder were obtained from national research center, Cairo Egypt. The powder was dissolved in distilled water prior to rat's administration.

Study protocol

Rats were divided into four groups, 10 rats each and classified as follows:

i. Control: rats were administered only distilled water as vehicle. ii. Lead: rats were given lead acetate (30 mg/kg b.w) of 1% solution per os. iii. Lead + *C. vulgaris*: rats were concomitantly orally received Lead acetate and *C. vulgaris* (300mg/Kg bw) by gavage tube. iv. Lead + *S. platensis*: rats were simultaneously orally treated with Lead and *S. platensis* (300mg/Kg bw). The experiment was continued for eight weeks.

Blood and testicular tissue processing

After eight weeks, each rat was weighted for determination of body weight gain. Then, rats were euthanized for blood samples compilation and Sera were harvested and stored at -20 °C for enzymes and hormonal analysis. Afterthought, rats were decapitated and both testes, epididymis, seminal vesicles, prostate glands were dissected and weighted. Gonadosomatic index (GSI) was calculated by dividing gonad weight with body weight and then multiplying with 100, where gonad weight = (weight of the right testis + weight of the left testis)/2 (Latif et al. 2008). Next, one testis was homogenized in phosphate-buffered saline (PBS), pH = 7.4 via a Teflon homogenizer, then harvested filtrate used for assessment of oxidative stress, inflammatory and apoptosis markers. Additionally, another testis was prepared for histological and immunohistochemical screening.

Semen characters

The caudal part of epididymis was cut and squeezed in order to harvesting the epididymal sperms. The concentration of sperms was carried post dilution of epididymal content by sodium citrate dihydrate

solution (2.9%) (Turk et al. 2007). Then, sperm abnormalities were assessed via slide smearing one drop of epididymal content, and then stained by Eosin Nigrosin (Okamura et al. 2005).

Sera analysis

Serum testicular enzymes activities and sex hormones levels

Sorbitol Dehydrogenase (SDH) activity is measured via ELISA kits of (KAMIYA BIOMEDICAL, Catalog# KT-28604). Acid phosphatase (ACP) activity is measured calorimetrically via kit of (DIACHEM Ltd, Catalog# 42401). Regarding, sex hormones levels were assessed in serum by the help of ELISA kit, Testosterone (CUSABIO, Catalog# CSB-E05100r), Follicular stimulating hormone (FSH) (CUSABIO, Catalog# CSB-E06869r), Luteinizing hormone (LH) (CUSABIO, Catalog# CSB- E12654r) according to the manufacturer's instructions.

Testicular antioxidant/oxidant, inflammatory, apoptosis biomarkers

Total antioxidative capacity (TAC) and total oxidative capacity (TOC) were measured Calorimetrically via kits of (Labor Diagnostika Nord GmbH& Co. KG, Catalog# OX 20-4100) and (Labor Diagnostika Nord GmbH& Co. KG, Catalog# OX 20-4200) respectively. Tumor necrosis factor (TNF-α) and Caspase- 3 were analyzed via ELISA kit of (CUSABIO, Catalog# CSB-E11987r) and (KAMIYA BIOMEDICAL, Catalog# KT-9429) respectively following manufacturer's instructions.

Testicular histopathology

testicular specimens from study groups were immersed in 10% neutral buffered formalin, dehydrated in ascending concentrations of ethyl alcohol (70–100%) and then used to process formalin fixed paraffin-embedded (FFPE) sections. Further, one FFPE specimen was stained with [hematoxylin and eosin (Hx&E)] for microscopic examination and imaged each section at 4x and 40x magnification.

Testicular immunohistochemistry (IHC)

IHC protocol was implemented following the method of (Williams et al. 2015). Formalin fixed paraffin embedded specimens were cut into 4-µm sections. After de-paraffinization, sections were heated in Tris/HCL buffer (pH 9.0) for 20 min at room temperature for antigen retrieval. Afterthought, sections were incubated with 0.3% H_2O_2 in absolute methanol for 30 minutes. Then, incubation with primary antibody for androgen receptor (Thermo Fisher Scientific Co., USA, Catalog# MA1-150), and secondary antibodies

as Biotinylated polyvalent (Thermo Scientific Co., UK, Catalog# 32230) were performed. All slides are lightly counterstained with hematoxylin for 30 seconds prior to dehydration and mounting, and imaged each section at 10x and 40x magnification. Testicular positively stained area percentage was quantified via ImageJ software per slide after light background subtraction.

Statistical analysis

Values were analyzed by the help of SPSS version 20. Results were expressed as mean± SE. One-way Analysis of Variance (ANOVA) was conducted for comparison between study groups followed by Duncan Multiple Rang Test (post hoc test). Significance was recorded at $P<0.01$.

Results

Effect of *C. vulgaris* or *S. platensis* supplementation on body weight gain and accessory reproductive organs weights in lead acetate exposed rats

Lead acetate- intoxicated rats demonstrated significantly ($P < 0.01$) reduction in body weight gain, weights of the testis, epididymis, prostate gland, seminal vesicle, and gonadosomatic index versus control. On contrary, Co-therapy of *C. vulgaris* or *S. platensis* (300 mg/kg b.w) with lead acetate for eight weeks restored wight gain, and sex organs weights (Table 1,2).

Effect of *C. vulgaris* or *S. platensis* supplementation on semen characters in lead acetate exposed rats

In comparison to control, there was lower sperm count in line with elevation in percentage of total malformation sperms in lead acetate exposed rats. While, *C. vulgaris* or *S. platensis* supplementation with lead acetate reversed the seminal alterations (Table 3).

Effect of *C. vulgaris* or *S. platensis* supplementation on serum testicular enzymes activities sex hormones levels in lead acetate exposed rats

Oral exposure of lead acetate (30 mg/kg b.w) promoted a significant ($p < 0.01$) elevation in ACP and SDH activities, minimized testosterone along with maximized FSH, LH levels in sera comparing to control. So, reduced sex organ weight, and seminal alterations are indicative for testosterone role in normal growth of sex organs and spermatogenesis. On the opposite, *C. vulgaris* or *S. platensis* supplementation with lead acetate reversed the previous deteriorations (Table 4).

Effect of *C. vulgaris* or *S. platensis* supplementation on testicular antioxidant/oxidant, inflammatory, apoptosis biomarkers in lead acetate exposed rats

Oral exposure of lead acetate fostered testicular oxidative, inflammatory burden, and apoptotic cascade as expressed by significantly ($p< 0.01$) TAC downregulation with TOC, TNF-α, and Caspase-3 levels upregulation versus control. In highly contrast, combined treating of *C. vulgaris* or *S. platensis* supplementation with lead acetate revealed antioxidant, anti-inflammatory and antiapoptotic potency (Table 5).

Testicular histopathological findings

Hx&E testicular sections of control group delineated seminiferous tubules (ST) with well-defined regular basement membrane and germinal epithelium. In addition to presence of regularly arranged Spermatogenic cells and uniform maturation of spermatozoa formation in ST. As well as, normally organized Leydig cells are seen (Figure 1(A, B)). In lead acetate intoxicated testis revealed that ST suffered from thinned irregular basement membrane, apparent disarrangement and damage. In addition to Spermatogenesis distribution with no evidence of spermatozoa formation and hyperplasia of Leydig cells (Figure 1(C, D)).

On the opposite, testicular section from *C. vulgaris* treated rats exhibited that ST are regular in shape and outline, and closely backed. Spermatogenesis is maintained in some tubules (50% of tubules), while others show vacuolization with evidence of disturbed spermatogenesis in line with absence of spermatozoa formation. Moreover, interstetium show Leydig cell hyperplasia (Figure 1(E, F)).

Regarding *S. platensis* supplemented rats, Seminiferous tubules are evenly spaced, uniform in shape, with regular outlines. Uniform spermatogenesis and spermatozoa formation are evident in most of tubules. In addition to, diminution of Leydig cells hyperplasia (Figure 1(G, H)). Collectively, supplementation of *S. platensis* significantly reversed the normal testicular histology with dynamic spermatogenesis implying the antioxidant, anti-inflammatory, antiapoptotic potency

Testicular immunohistochemistry

Nuclear AR expression pattern was strong in Spermatogenic cells and Leydig cells in control group (Figure 2I (A, B)). However, lead acetate group showed weak staining intensity of the expression area of AR in nuclei of Spermatogenic cells and moderate staining intensity in Leydig cells (Figure 2 I (C, D)).

Combined treating of *C. vulgaris* with lead acetate was able to moderately restore AR testicular loss as outlined by moderate expression of AR in spermatogenic cells in uniform tubule. While in injured tubules

there is weak expression of AR in nuclei of remaining spermatogenic cells. There is strong AR expression in Leydig cells (Figure 2I (E, F)).

Furthermore, co- therapy of *S. platensis* with lead acetate can restore normal AR testicular expression as pronounced by moderate to strong nuclear expression of AR in nuclei of Spermatogenic cell. As well as, there is strong AR expression in Leydig cells (Figure 2I (G, H)).

Quantification of these findings pronounced that administration of lead acetate significantly downregulated AR expression versus control rats. However, combined therapy of *S. platensis* or C. vulgaris with lead acetate significantly upregulated AR expression comparing to lead acetate treated rats with the best ameliorative effect for *S. platensis* (Figure 2II).

Discussion

Lead is one of male reproductive toxicant in humans and animals elicits testicular dysfunction and infertility (Al-Megrin et al. 2020).

In the running study, lead acetate prompted reduction in body weight gain comparing to control that may contribute to imbalance metabolism due to impairing zinc level whereby required for metabolic processes (Ademuyiwa et al. 2010). Or lead acetate may decrease feed intake (Winiarska-Mieczan et al. 2018).

Additionally, fall in reproductive organ weights, GSI, sperm cell count, and elevation in sperm malformation percentage were recorded in lead acetate treated rats. Our findings came in harmony with (Vidal and Whitney 2014) who demonstrated that testicular weight is correlated with the differentiated spermatogenic cells mass. Thereby, lessened GSI implying loss of germ cell. Importantly, lead accumulation in reproductive organs generated ROS altering steroidogenesis and spermatogenesis (Jegede et al. 2015). Also, lead lessened sperm calcium and cyclic adenosine monophosphate levels lowering tyrosine phosphorylation of protein and hindering sperm impairment (He et al. 2016). We herein explored that lead acetate significantly elevated ACP and SDH activity in sera versus control. ACP is secreted by Sertoli cells and detected in lysosomes of Leydig cells mediated lysis of abnormal sperms (Olayinka and Ore 2015). While, SDH is distributed in spermatogenic cells of seminiferous tubules and correlated with germ cell maturation via energy supply by conversion of sorbitol to fructose (Tripathi et al. 2016). Remarkably, Lead acetate has been evidenced in testicular accumulation with subsequent upregulation of reactive oxygen species (ROS) generation along with antioxidants downregulation promoting cellular membrane harm via lipid peroxidation (El-Khadragy et al. 2020). ROS- induced cellular injury and could be associated with enzyme liberation into the circulation (Hassan et al. 2013). Hence, elevated serum ACP and SDH implying testicular oxidative burden promoted by lead acetate.

Concerning hormonal profile, lead acetate disrupted steroidogenesis confirmed by minimized serum testosterone levels along with maximized FSH, LH levels, and downregulation in nuclear AR expression in spermatogenic cells and Leydig cells versus control. Fall in testosterone level can attribute to lead-promoted testicular oxidative and immune response consistently with (Allouche et al. 2009; Udefa et al.

2020). These are similarly to our findings reveal decline in testicular TAC with elevation in TOC, and TNF-α levels. These may account to Lead acetate counteracted antioxidant enzymes activities through targeting SH groups and/ or metal cofactors of antioxidants (Patra et al. 2011). As well as, lead acetate downregulated Nfe212 gene expression- promoted downregulation of antioxidant enzymes expression (Kabel and Elkhoely 2017).The resulted testicular oxidative response is proposed as prospective pathway for immune response via TNF-α expression by macrophages post lead acetate exposure (Salama et al. 2016). These resulting in steroidogenesis inhibition in Leydig cells whereby TNF-α lessened testicular 3b- and 17b-17ß-hydroxysteroid dehydrogenase activity resulting in reduced testosterone level (Hales 2002; Hong et al. 2004). Consequently, AR damage are occurred in response to testicular oxidative burden (Chang et al. 2019; Yao et al. 2007).

Next, AR possessed a fundamental role in testosterone feedback regulation via autocrine action on Leydig cells, through decreasing Gonadotropin-releasing hormone (GnRH) resulted in pituitary LH inhibition (Amory and Bremner 2001). Further, downregulation of AR expression in high fat diet supplemented mice was associated with minimization in testosterone level and infertility (Fan et al. 2015).Therefore, regressed AR expression may be raised LH and FSH release to compensate the testicular disruptions and testosterone depletion.

Furthermore, TNF-α- mediated disruption of mitochondrial membrane that accompanied by cytochrome C release, caspase-9, and Caspase-3 activation mediated apoptosis (Zhou et al. 2019). Moreover, Lead acetate in spermatogenic cells upregulated caspase-3, and Bax in line with downregulated Bcl2 (Hassan et al. 2019). Consequently, further germ cells apoptosis, sperm membrane perturbation and DNA fragmentation are detected (Henkel et al. 2010). Currently, these findings were confirmed by the testicular architectural deteriorations that revealed irregular shape of seminiferous tubules with no evidence of spermatozoa formation and Leydig cell hyperplasia.

On the contrary, co-therapy of lead acetate with *C. vulgaris* or *S. platensis* significantly attenuated lead acetate promoted testicular dysfunction.

Concerning the reproductive defensive mechanism of *C. vulgaris* is supported by our data such restoration of weight gain, sex organ weight, serum ACP, SDH activity, testosterone, FSH, LH levels, sperm cell count, and lessened sperm malformation percentage and upregulation of testicular AR expression. AS well as, upregulation in testicular TAC, downregulation in TOC, TNF-α and Caspase-3 expression were recorded agreeing with (Abdelhamid et al. 2020; Mustafa 2015; Raj et al. 2013; Vijayavel et al. 2007). These findings emphasized many previously published researches. Feeding of *C. vulgaris* enriched diet 0, 3 or 5% upregulated liver and plasma antioxidant enzymes in cadmium- exposed rats for 10 weeks (Son et al. 2009). Moreover, *C. vulgaris* (150 mg/kg b.w) ameliorated lipid peroxidation, DNA damage, TNF-α expression, as well as restored antioxidant enzymes in STZ diabetic rats (Aizzat et al. 2010). Furthermore, *C. vulgaris* supplementation (50 mg/kg bw) counteracted aging- promoted oxidative stress in C57BL/6 mice for eight weeks (Aliahmat et al. 2012). Similarly, (Mustafa 2015) proposed that co-treating of *C. vulgaris* (50 mg/kg bw) with lead acetate 200 mg/l for 12 weeks was able to restore thickness of

germinal epithelium diameter of seminiferous tubules and, and sperm structure that is due to its chelating and antioxidant potential. Recently, *C. vulgaris* dietary supplementation upregulated hepatic and ovarian antioxidants gene expression, as well as body performance enhancement in New Zealand White rabbits (Sikiru et al. 2019).

These curative effects may attribute to its antioxidant content such as lycopene, eicosatetraenoic, docosahexaenoic acid, astaxanthin, polysaccharides, polyphenols, lutein and fucoxanthin, omega 3, omega 6, reduced glutathione, chlorophyll, and vitamin C, E (Rahimnejad et al. 2017; Renju et al. 2014; Vijayavel et al. 2007) highlighting its direct ROS scavenging properties. Accordingly, lycopene upregulated the epididymal AR expression in Polychlorinated Biphenyls–intoxicated rats (Raj et al. 2013). Eicosapentaenoic and docosahexaenoic acid counteracted cisplatin-promoted testicular and seminal toxicity in rats (Ciftci et al. 2014). Further, astaxanthin ameliorated testicular and sex hormone alterations in high fructose supplemented rats (Dokumacioglu et al. 2018). In vitro and in vivo, *C. vulgaris* polysaccharides evoked antioxidant possibilities via scavenging superoxide, DPPH and hydroxyl radical-scavenging (Yu et al. 2019). Furthermore, omega-3 fatty acids pronounced antioxidant and anti-inflammatory activities in a rat model of stress-induced liver injury (Ali and Rifaai 2019).

In this context, oral *S. platensis* administration reserved weight gain and reproductive organ weights that may relate to direct lead adsorption by *S. platensis* favoring rapid body lead elimination (Banji et al. 2013). As well as, *S. platensis* nutritional ingredient such protein, essential fatty acids, vitamins, iron, zinc, polysaccharides, phenols, carotenoids, chlorophyll, C-Phycocyanin pigment are enriched the body (Hosseini et al. 2013).

More confirmation in our work, *S. platensis* alleviated serum ACP, SDH activity, testosterone, FSH, LH alterations, sperm cell count, and lessened sperm malformation percentage. Also, it upregulated testicular AR, TAC along with downregulated TOC, TNF-α, Caspase-3 expression agreeing with many previous researches (Abdel-Daim et al. 2019; Bashandy et al. 2016; Esener et al. 2017; Sadek et al. 2017). Oral pretreatment with *S. platensis* (500 and 1000 mg/kg bw) one hour prior to deltamethrin exposure for five days improved hepatic and renal antioxidants prospect (Abdel-Daim et al. 2013). Moreover, twice weekly oral supplementation of *S. platensis* (500 mg/kg b.w) for eight weeks exhibited hepatic and pancreatic β-cells antioxidant, anti-inflammatory, and anti-apoptotic potential in STZ- diabetic rats (Sadek et al. 2017). Furthermore, administration of *S. platensis* (300 mg/kg bw) for four weeks could alleviate sperm quality and sex hormone alterations in furan treated rats (Abd El-Hakim et al. 2018). Similarly, another strain, *S. maxima* was able to up reregulate testicular antioxidant enzymes and downregulate caspase-3 in lead acetate-exposed rats (Abdrabou et al. 2019).

These ameliorative events may attribute to antioxidant active ingredients of *S. platensis*. Phenols scavenged ROS and improved testicular antioxidant status in alloxan-diabetic rats (Roy et al. 2015). Specifically, C-phycocyanin is detected in Spirulina only and revealed antioxidant and antiapoptotic potentials in cardiomyocytes of doxorubicin- treated rats (Khan et al. 2006), and in paraquat- promoted acute lung injury in rats (Sun et al. 2011). Chlorophyll evoked counteracted sodium nitrate- induced renal

and hepatic oxidative burden in rats (Suparmi et al. 2016). Moreover, vitamin C, E influenced testicular antioxidant attributes versus lead in rats (Ayinde et al. 2012).These are consolidated by architectural restoration as evident by presence of uniform Seminiferous tubules and spermatozoa.

Therefore, the antioxidant and anti-inflammatory potency of *C. vulgaris* and *S. platensis* can reverse lead acetate- fostered oxidants, TNF-α, and caspase 3 expression- mediated testicular apoptosis, AR damage and steroidogenesis inhibition in Leydig cells. Hence, testosterone and AR restoration boosted spermatogenesis are expressed by remarkably raised sperm count and sex organ weight.

Conclusion

The above-mentioned data can consolidate the protective potential of oral supplementation of *C. vulgaris* or *S. platensis* (300 mg/kg bw) for 8 weeks in modulating lead acetate-boosted testicular dysfunction via antioxidant, immunomodulatory, anti-apoptotic potentials enhancing testicular cells, and androgen receptor restoration with superior impacts to *S. platensis* comparing to *C. vulgaris*. Totally, regenerated testicular cells can restore steroidogenesis producing testosterone that mediated spermatogenesis in seminiferous tubules with respect to AR.

Abbreviations

(GSI), gonadosomatic index; (FSH), follicle-stimulating hormone; (LH), luteinizing hormone; (ACP), Acid phosphatase; (SDH), Sorbitol Dehydrogenase; (TAC), total antioxidative capacity; (TOC), total oxidative capacity; (TNF-α), tumor necrosis factor alpha; (AR), androgen receptor.

Declarations

Author information

Affiliations

Department of Biochemistry, Faculty of Vet. Medicine, Suez Canal University Ismailia 41522, Egypt

Ibrahim A. Ibrahim, Abeir A. Shalaby, Raghda T. Abd Elaziz, Hoda I. Bahr

Author Contributions

Idea and protocol design: I.A.I., A.A.S., and H.I.B. Methodology and experimentation: I.A.I., A.A.S., R.T.A.E. and H.I.B. Data analysis: I.A.I., A.A.S., R.T.A.E. and H.I.B. Funding: R.T.A.E. All authors shared draft writing. All authors approved the submission. Corresponding author

Correspondence to Hoda I. Bahr

Data availability statement

All required data will be available with the corresponding author upon request.

Compliance with ethical standards

Conflict of interest statement

The authors declare that they have no conflict of interest.

Consent for publication

All authors approve this submission.

Consent to participate

Not applicable as the study did not include human subject.

Ethics approval

Study protocol was complied with the ethical guidelines for laboratory animals use at the Faculty of Veterinary Medicine, Suez Canal University, Egypt (approval NO.).

References

1. Abd El-Hakim YM, Mohamed WA, El-Metwally AE (2018) Spirulina platensis attenuates furan reprotoxicity by regulating oxidative stress, inflammation, and apoptosis in testis of rats Ecotoxicol Environ Saf 161:25-33 doi:10.1016/j.ecoenv.2018.05.073
2. Abdel-Daim MM, Abuzead SM, Halawa SM (2013) Protective role of Spirulina platensis against acute deltamethrin-induced toxicity in rats PLoS One 8:e72991 doi:10.1371/journal.pone.0072991
3. Abdel-Daim MM et al. (2019) Influence of Spirulina platensis and ascorbic acid on amikacin-induced nephrotoxicity in rabbits Environ Sci Pollut Res Int 26:8080-8086 doi:10.1007/s11356-019-04249-4
4. Abdel-Daim MM et al. (2020) Spirulina platensis mediated the biochemical indices and antioxidative function of Nile tilapia (Oreochromis niloticus) intoxicated with aflatoxin B1 Toxicon 184:152-157 doi:10.1016/j.toxicon.2020.06.001
5. Abdel-Daim MM, Farouk SM, Madkour FF, Azab SS (2015) Anti-inflammatory and immunomodulatory effects of Spirulina platensis in comparison to Dunaliella salina in acetic acid-

induced rat experimental colitis Immunopharmacology and Immunotoxicology 37:126-139 doi:10.3109/08923973.2014.998368

6. Abdel Daim M (2014) Pharmacodynamic interaction of Spirulina platensis with erythromycin in Egyptian Baladi bucks (Capra hircus) Small Ruminant Research 120:234-241
7. Abdelhamid FM, Elshopakey GE, Aziza AE (2020) Ameliorative effects of dietary Chlorella vulgaris and beta-glucan against diazinon-induced toxicity in Nile tilapia (Oreochromis niloticus) Fish Shellfish Immunol 96:213-222 doi:10.1016/j.fsi.2019.12.009
8. Abdelkhalek NKM, Ghazy EW, Abdel-Daim MM (2015) Pharmacodynamic interaction of Spirulina platensis and deltamethrin in freshwater fish Nile tilapia, Oreochromis niloticus: impact on lipid peroxidation and oxidative stress Environmental Science and Pollution Research 22:3023-3031 doi:10.1007/s11356-014-3578-0
9. Abdrabou MI, Elleithy EMM, Yasin NAE, Shaheen YM, Galal M (2019) Ameliorative effects of Spirulina maxima and Allium sativum on lead acetate-induced testicular injury in male albino rats with respect to caspase-3 gene expression Acta Histochem 121:198-206 doi:10.1016/j.acthis.2018.12.006
10. Adela Y, Ambelu A, Tessema DA (2012) Occupational lead exposure among automotive garage workers - a case study for Jimma town, Ethiopia J Occup Med Toxicol 7:15 doi:10.1186/1745-6673-7-15
11. Ademuyiwa O, Agarwal R, Chandra R, Behari JR (2010) Effects of sub-chronic low-level lead exposure on the homeostasis of copper and zinc in rat tissues J Trace Elem Med Biol 24:207-211 doi:10.1016/j.jtemb.2010.01.002
12. Aizzat O et al. (2010) Modulation of oxidative stress by Chlorella vulgaris in streptozotocin (STZ) induced diabetic Sprague-Dawley rats Advances in medical sciences 55:281-288
13. Al-Megrin WA et al. (2020) Luteolin protects against testicular injury induced by lead acetate by activating the Nrf2/HO-1 pathway IUBMB Life 72:1787-1798 doi:10.1002/iub.2311
14. Aladaileh SH et al. (2020) Spirulina platensis ameliorates the sub chronic toxicities of lead in rabbits via anti-oxidative, anti- inflammatory, and immune stimulatory properties Science of The Total Environment 701:134879 doi:https://doi.org/10.1016/j.scitotenv.2019.134879
15. Ali FF, Rifaai RA (2019) Preventive effect of omega-3 fatty acids in a rat model of stress-induced liver injury Journal of cellular physiology 234:11960-11968 doi:10.1002/jcp.27848
16. Ali N et al. (2020) Arsenic and lead in the indoor residential settings of different socio-economic status; assessment of human health risk via dust exposure Environ Sci Pollut Res Int doi:10.1007/s11356-020-11546-w
17. Aliahmat NS, Noor MR, Yusof WJ, Makpol S, Ngah WZ, Yusof YA (2012) Antioxidant enzyme activity and malondialdehyde levels can be modulated by Piper betle, tocotrienol rich fraction and Chlorella vulgaris in aging C57BL/6 mice Clinics (Sao Paulo, Brazil) 67:1447-1454 doi:10.6061/clinics/2012(12)16

18. Allouche L, Hamadouche M, Touabti A (2009) Chronic effects of low lead levels on sperm quality, gonadotropins and testosterone in albino rats Exp Toxicol Pathol 61:503-510 doi:10.1016/j.etp.2008.12.003
19. Amory JK, Bremner W (2001) Endocrine regulation of testicular function in men: implications for contraceptive development Mol Cell Endocrinol 182:175-179 doi:10.1016/s0303-7207(01)00562-7
20. Ayinde OC, Ogunnowo S, Ogedegbe RA (2012) Influence of Vitamin C and Vitamin E on testicular zinc content and testicular toxicity in lead exposed albino rats BMC Pharmacol Toxicol 13:17 doi:10.1186/2050-6511-13-17
21. Banji D, Banji OJ, Pratusha NG, Annamalai AR (2013) Investigation on the role of Spirulina platensis in ameliorating behavioural changes, thyroid dysfunction and oxidative stress in offspring of pregnant rats exposed to fluoride Food Chem 140:321-331 doi:10.1016/j.foodchem.2013.02.076
22. Barkallah M, Slima AB, Elleuch F, Fendri I, Pichon C, Abdelkafi S, Baril P (2020) Protective Role of Spirulina platensis Against Bifenthrin-Induced Reprotoxicity in Adult Male Mice by Reversing Expression of Altered Histological, Biochemical, and Molecular Markers Including MicroRNAs Biomolecules 10:753 doi:10.3390/biom10050753
23. Bashandy SA, El Awdan SA, Ebaid H, Alhazza IM (2016) Antioxidant Potential of Spirulina platensis Mitigates Oxidative Stress and Reprotoxicity Induced by Sodium Arsenite in Male Rats Oxidative medicine and cellular longevity 2016:7174351 doi:10.1155/2016/7174351
24. Bauer LM, Costa JAV, da Rosa APC, Santos LO (2017) Growth stimulation and synthesis of lipids, pigments and antioxidants with magnetic fields in Chlorella kessleri cultivations Bioresource Technology 244:1425-1432 doi:https://doi.org/10.1016/j.biortech.2017.06.036
25. Blas-Valdivia V, Ortiz-Butrón R, Pineda-Reynoso M, Hernández-Garcia A, Cano-Europa E (2010) Chlorella vulgaris administration prevents HgCl2-caused oxidative stress and cellular damage in the kidney Journal of Applied Phycology 23:53-58 doi:10.1007/s10811-010-9534-6
26. Camacho F, Macedo A, Malcata F (2019) Potential Industrial Applications and Commercialization of Microalgae in the Functional Food and Feed Industries: A Short Review Mar Drugs 17 doi:10.3390/md17060312
27. Chang XR, Yao YL, Wang D, Ma HT, Gou PH, Li CY, Wang JL (2019) Influence of hypothyroidism on testicular mitochondrial oxidative stress by activating the p38 mitogen-activated protein kinase and c-Jun NH2-terminal kinase signaling pathways in rats Human & experimental toxicology 38:95-105 doi:10.1177/0960327118781927
28. Ciftci O, Cetin A, Aydin M, Kaya K, Oguz F (2014) Fish oil, contained in eicosapentaenoic acid and docosahexaenoic acid, attenuates testicular and spermatological damage induced by cisplatin in rats Andrologia 46:1161-1168 doi:10.1111/and.12209
29. Czerwonka A et al. (2018) Anticancer effect of the water extract of a commercial Spirulina (Arthrospira platensis) product on the human lung cancer A549 cell line Biomedicine & Pharmacotherapy 106:292-302 doi:https://doi.org/10.1016/j.biopha.2018.06.116

30. Dokumacioglu E et al. (2018) Effects of astaxanthin on biochemical and histopathological parameters related to oxidative stress on testes of rats on high fructose regime Andrologia 50:e13042 doi:10.1111/and.13042
31. El-Khadragy M et al. (2020) Impact of Coenzyme Q10 Administration on Lead Acetate-Induced Testicular Damage in Rats Oxid Med Cell Longev 2020:4981386 doi:10.1155/2020/4981386
32. Esener OBB et al. (2017) Spirulina Platensis Affects Factors Involved in Spermatogenesis and Increases Ghrelin Receptors in Testis Tissue of Rats Fed a High-Fat Diet Polish journal of veterinary sciences 20:467-475 doi:10.1515/pjvs-2017-0056
33. Fan Y, Liu Y, Xue K, Gu G, Fan W, Xu Y, Ding Z (2015) Diet-Induced Obesity in Male C57BL/6 Mice Decreases Fertility as a Consequence of Disrupted Blood-Testis Barrier PLOS ONE 10:e0120775 doi:10.1371/journal.pone.0120775
34. Gargouri M, Magné C, El Feki A (2016) Hyperglycemia, oxidative stress, liver damage and dysfunction in alloxan-induced diabetic rat are prevented by Spirulina supplementation Nutrition Research 36:1255-1268 doi:https://doi.org/10.1016/j.nutres.2016.09.011
35. Goto Y et al. (2020) Association of prenatal maternal blood lead levels with birth outcomes in the Japan Environment and Children's Study (JECS): a nationwide birth cohort study Int J Epidemiol doi:10.1093/ije/dyaa162
36. Hales DB (2002) Testicular macrophage modulation of Leydig cell steroidogenesis J Reprod Immunol 57:3-18 doi:10.1016/s0165-0378(02)00020-7
37. Hassan E, El-Neweshy M, Hassan M, Noreldin A (2019) Thymoquinone attenuates testicular and spermotoxicity following subchronic lead exposure in male rats: Possible mechanisms are involved Life Sci 230:132-140 doi:10.1016/j.lfs.2019.05.067
38. Hassan HA, Isa AM, El-Kholy WM, Nour SE (2013) Testicular disorders induced by plant growth regulators: cellular protection with proanthocyanidins grape seeds extract Cytotechnology 65:851-862 doi:10.1007/s10616-012-9525-3
39. Haw KY, Chakravarthi S, Haleagrahara N, Rao M (2012) Effects of Etlingera elatior extracts on lead acetate-induced testicular damage: A morphological and biochemical study Exp Ther Med 3:99-104 doi:10.3892/etm.2011.355
40. Hazra R, Jimenez M, Desai R, Handelsman DJ, Allan CM (2013) Sertoli cell androgen receptor expression regulates temporal fetal and adult Leydig cell differentiation, function, and population size Endocrinology 154:3410-3422 doi:10.1210/en.2012-2273
41. He Y, Zou Q, Chen H, Weng S, Luo T, Zeng X (2016) Lead Inhibits Human Sperm Functions by Reducing the Levels of Intracellular Calcium, cAMP, and Tyrosine Phosphorylation The Tohoku journal of experimental medicine 238:295-303 doi:10.1620/tjem.238.295
42. Henkel R, Bastiaan HS, Schüller S, Hoppe I, Starker W, Menkveld R (2010) Leucocytes and intrinsic ROS production may be factors compromising sperm chromatin condensation status Andrologia 42:69-75 doi:10.1111/j.1439-0272.2009.00967.x

43. Hong CY et al. (2004) Molecular mechanism of suppression of testicular steroidogenesis by proinflammatory cytokine tumor necrosis factor alpha Mol Cell Biol 24:2593-2604 doi:10.1128/mcb.24.7.2593-2604.2004
44. Hosseini SM, Khosravi-Darani K, Mozafari MR (2013) Nutritional and medical applications of spirulina microalgae Mini reviews in medicinal chemistry 13:1231-1237 doi:10.2174/1389557511313080009
45. Ibrahim AE, Abdel-Daim MM (2015) Modulating Effects of Spirulina platensis against Tilmicosin-Induced Cardiotoxicity in Mice Cell journal 17:137-144 doi:10.22074/cellj.2015.520
46. Jegede AI, Offor U, Azu OO, Akinloye O (2015) Red Palm Oil Attenuates Lead Acetate Induced Testicular Damage in Adult Male Sprague-Dawley Rats Evid Based Complement Alternat Med 2015:130261 doi:10.1155/2015/130261
47. Kabel AM, Elkhoely AA (2017) Ameliorative Effect of Coenzyme Q10 and/or Candesartan on Carboplatin-Induced Nephrotoxicity: Roles of Apoptosis, Transforming Growth Factor-Beta1, Nuclear Factor Kappa-B And The Nrf2/HO-1 Pathway Asian Pacific journal of cancer prevention : APJCP 18:1629-1636 doi:10.22034/APJCP.2017.18.6.1629
48. Kata FS, Athbi AM, Manwar EQ, Al-Ashoor A, Abdel-Daim MM, Aleya L (2018) Therapeutic effect of the alkaloid extract of the cyanobacterium Spirulina platensis on the lipid profile of hypercholesterolemic male rabbits Environmental Science and Pollution Research 25:19635-19642 doi:10.1007/s11356-018-2170-4
49. Khan M, Varadharaj S, Shobha JC, Naidu MU, Parinandi NL, Kutala VK, Kuppusamy P (2006) C-phycocyanin ameliorates doxorubicin-induced oxidative stress and apoptosis in adult rat cardiomyocytes J Cardiovasc Pharmacol 47:9-20 doi:10.1097/01.fjc.0000191520.48404.27
50. Mokhtari M, Zanboori M (2011) The effects of lead acetate on sexual behavior and the level of testosterone in adult male rats Int J Fertil Steril 5:13-20
51. Mustafa HN (2015) Potential Alleviation of Chlorella vulgaris and Zingiber officinale on Lead-Induced Testicular Toxicity: an Ultrastructural Study Folia Biol (Krakow) 63:269-278 doi:10.3409/fb63_4.269
52. Oduwole OO, Peltoketo H, Huhtaniemi IT (2018) Role of Follicle-Stimulating Hormone in Spermatogenesis Front Endocrinol (Lausanne) 9:763 doi:10.3389/fendo.2018.00763
53. Offor SJ, Mbagwu HOC, Orisakwe OE (2017) Lack of beneficial effect of activated charcoal in lead induced testicular toxicity in male albino rats Middle East Fertility Society Journal 22:189-192 doi:10.1016/j.mefs.2017.02.001
54. Okamura A et al. (2005) A comprehensive evaluation of the testicular toxicity of dichlorvos in Wistar rats Toxicology 213:129-137 doi:10.1016/j.tox.2005.05.015
55. Olayinka ET, Ore A (2015) Hepatotoxicity, Nephrotoxicity and Oxidative Stress in Rat Testis Following Exposure to Haloxyfop-p-methyl Ester, an Aryloxyphenoxypropionate Herbicide Toxics 3:373-389 doi:10.3390/toxics3040373
56. Patra RC, Rautray AK, Swarup D (2011) Oxidative stress in lead and cadmium toxicity and its amelioration Vet Med Int 2011:457327-457327 doi:10.4061/2011/457327

57. Rahimnejad S, Lee S-M, Park H-G, Choi J (2017) Effects of Dietary Inclusion ofChlorella vulgarison Growth, Blood Biochemical Parameters, and Antioxidant Enzyme Activity in Olive Flounder,Paralichthys olivaceus Journal of the World Aquaculture Society 48:103-112 doi:10.1111/jwas.12320
58. Raj M, Kandaswamy D, Krishnamoorthy G, Revathy S, Perumal E, Arunakaran J (2013) Impact of Lycopene on Epididymal Androgen and Estrogen Receptors' Expression in Polychlorinated Biphenyls-Exposed Rat Reproductive sciences (Thousand Oaks, Calif) 21 doi:10.1177/1933719113492213
59. Renju GL, Muraleedhara Kurup G, Bandugula VR (2014) Effect of lycopene isolated from Chlorella marina on proliferation and apoptosis in human prostate cancer cell line PC-3 Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine 35:10747-10758 doi:10.1007/s13277-014-2339-5
60. Roy VK, Chenkual L, Gurusubramanian G (2015) Protection of testis through antioxidant action of Mallotus roxburghianus in alloxan-induced diabetic rat model Journal of ethnopharmacology 176:268-280 doi:10.1016/j.jep.2015.11.006
61. Sadek KM, Lebda MA, Nasr SM, Shoukry M (2017) Spirulina platensis prevents hyperglycemia in rats by modulating gluconeogenesis and apoptosis via modification of oxidative stress and MAPK-pathways Biomed Pharmacother 92:1085-1094 doi:10.1016/j.biopha.2017.06.023
62. Sajilata MG, Singhal RS, Kamat MY (2008) Fractionation of lipids and purification of γ-linolenic acid (GLA) from Spirulina platensis Food Chemistry 109:580-586 doi:10.1016/j.foodchem.2008.01.005
63. Salama SA, Arab HH, Maghrabi IA, Hassan MH, AlSaeed MS (2016) Gamma-Glutamyl Cysteine Attenuates Tissue Damage and Enhances Tissue Regeneration in a rat Model of Lead-Induced Nephrotoxicity Biological trace element research 173:96-107 doi:10.1007/s12011-016-0624-4
64. Sikiru AB, Arangasamy A, Alemede IC, Guvvala PR, Egena SSA, Ippala JR, Bhatta R (2019) Chlorella vulgaris supplementation effects on performances, oxidative stress and antioxidant genes expression in liver and ovaries of New Zealand White rabbits Heliyon 5:e02470-e02470 doi:10.1016/j.heliyon.2019.e02470
65. Son YA, Shim JA, Hong S, Kim MK (2009) Intake of Chlorella vulgaris improves antioxidative capacity in rats oxidatively stressed with dietary cadmium Ann Nutr Metab 54:7-14 doi:10.1159/000199453
66. Souza Queiroz J et al. (2016) Chlorella vulgaris reduces the impact of stress on hypothalamic-pituitary-adrenal axis and brain c-fos expression Psychoneuroendocrinology 65:1-8 doi:10.1016/j.psyneuen.2015.12.002
67. Sukhn C, Awwad J, Ghantous A, Zaatari G (2018) Associations of semen quality with non-essential heavy metals in blood and seminal fluid: data from the Environment and Male Infertility (EMI) study in Lebanon J Assist Reprod Genet 35:1691-1701 doi:10.1007/s10815-018-1236-z
68. Sun Y, Zhang J, Yan Y, Chi M, Chen W, Sun P, Qin S (2011) The protective effect of C-phycocyanin on paraquat-induced acute lung injury in rats Environ Toxicol Pharmacol 32:168-174 doi:10.1016/j.etap.2011.04.008

69. Suparmi S, Fasitasari M, Martosupono M, Mangimbulude JC (2016) Comparisons of Curative Effects of Chlorophyll from Sauropus androgynus (L) Merr Leaf Extract and Cu-Chlorophyllin on Sodium Nitrate-Induced Oxidative Stress in Rats J Toxicol 2016:8515089 doi:10.1155/2016/8515089
70. Tripathi M, Singh AP, Gupta G, Rajender S (2016) Concomitant and discrete expressions of aldose reductase and sorbitol dehydrogenase in the male reproductive tract Acta Histochem 118:776-783 doi:10.1016/j.acthis.2016.09.008
71. Turk G, Atessahin A, Sonmez M, Yuce A, Ceribasi AO (2007) Lycopene protects against cyclosporine A-induced testicular toxicity in rats Theriogenology 67:778-785 doi:10.1016/j.theriogenology.2006.10.013
72. Udefa AL et al. (2020) Antioxidant, anti-inflammatory and anti-apoptotic effects of hydro-ethanolic extract of Cyperus esculentus L. (tigernut) on lead acetate-induced testicular dysfunction in Wistar rats Biomed Pharmacother 129:110491 doi:10.1016/j.biopha.2020.110491
73. Vijayavel K, Anbuselvam C, Balasubramanian MP (2007) Antioxidant effect of the marine algae Chlorella vulgaris against naphthalene-induced oxidative stress in the albino rats Mol Cell Biochem 303:39-44 doi:10.1007/s11010-007-9453-2
74. Wang H-M, Pan J-L, Chen C-Y, Chiu C-C, Yang M-H, Chang H-W, Chang J-S (2010) Identification of anti-lung cancer extract from Chlorella vulgaris C-C by antioxidant property using supercritical carbon dioxide extraction Process Biochemistry 45:1865-1872 doi:10.1016/j.procbio.2010.05.023
75. Wang RS, Yeh S, Tzeng CR, Chang C (2009) Androgen receptor roles in spermatogenesis and fertility: lessons from testicular cell-specific androgen receptor knockout mice Endocr Rev 30:119-132 doi:10.1210/er.2008-0025
76. Wang X, Zou P, He Y, Meng K, Quan F, Zhang Y (2018) Effect of luteinizing hormone on goat theca cell apoptosis and steroidogenesis through activation of the PI3K/AKT pathway Anim Reprod Sci 190:108-118 doi:10.1016/j.anireprosci.2018.01.014
77. Williams EM, Higgins JP, Sangoi AR, McKenney JK, Troxell ML (2015) Androgen receptor immunohistochemistry in genitourinary neoplasms Int Urol Nephrol 47:81-85 doi:10.1007/s11255-014-0834-7
78. Winiarska-Mieczan A, Kwiecien M, Kwiatkowska K, Kowalczuk-Vasilev E, Kiczorowska B (2018) Estimation of weekly intake of cadmium and lead by consumption of commercial ready-to-feed infant foods Food Addit Contam Part B Surveill 11:223-228 doi:10.1080/19393210.2018.1491644
79. Wu Q, Liu L, Miron A, Klimova B, Wan D, Kuca K (2016) The antioxidant, immunomodulatory, and anti-inflammatory activities of Spirulina: an overview Archives of toxicology 90:1817-1840 doi:10.1007/s00204-016-1744-5
80. Yao PL, Lin YC, Sawhney P, Richburg JH (2007) Transcriptional regulation of FasL expression and participation of sTNF-alpha in response to sertoli cell injury J Biol Chem 282:5420-5431 doi:10.1074/jbc.M609068200
81. Yu M et al. (2019) Preparation of Chlorella vulgaris polysaccharides and their antioxidant activity in vitro and in vivo Int J Biol Macromol 137:139-150 doi:10.1016/j.ijbiomac.2019.06.222

82. Zhou L, Wang S, Cao L, Ren X, Li Y, Shao J, Xu L (2019) Lead acetate induces apoptosis in Leydig cells by activating PPARgamma/caspase-3/PARP pathway Int J Environ Health Res:1-11 doi:10.1080/09603123.2019.1625034

Tables

Table 1

Effect of *Chlorella vulgaris* or *Spirulina platensis* supplementation on body weight gain, testes weight, and gonadosomatic index in lead acetate treated rats.

Groups	Weight gain (g)	Right testis (g)	Left testis (g)	Gonadosomatic index (%)
Control	$93.00^{a} \pm 2.60$	$1.62^{a} \pm 0.02$	$1.63^{a} \pm 0.02$	$0.43^{a} \pm 0.01$
Lead	$66.38^{c} \pm 1.71$	$1.23^{c} \pm 0.03$	$1.23^{c} \pm 0.02$	$0.34^{c} \pm 0.01$
Lead + C. vulgaris	$83.00^{b} \pm 1.72$	$1.37^{b} \pm 0.03$	$1.37^{b} \pm 0.03$	$0.39^{b} \pm 0.01$
Lead + S. platensis	$86.25^{b} \pm 1.84$	$1.47^{b} \pm 0.05$	$1.45^{b} \pm 0.04$	$0.40^{ab} \pm 0.01$
Within the same column, means ± SE with different superscripts is significantly ($P < 0.01$) differed.				

Table 2

Effect of *Chlorella vulgaris* or *Spirulina platensis* supplementation on accessory reproductive organs weight and seminal picture in lead acetate treated rats.

Groups	Tail of epididymis (g)	Prostate glands (g)	Seminal vesicles (g)
Control	$0.65^{a} \pm 0.03$	$0.94^{a} \pm 0.04$	$0.76^{a} \pm 0.03$
Lead	$0.22^{d} \pm 0.02$	$0.35^{c} \pm 0.01$	$0.32^{c} \pm 0.04$
Lead + C. vulgaris	$0.34^{c} \pm 0.02$	$0.64^{b} \pm 0.04$	$0.57^{b} \pm 0.05$
Lead + S. platensis	$0.45^{b} \pm 0.04$	$0.79^{a} \pm 0.05$	$0.68^{ab} \pm 0.05$
Within the same column, means ± SE with different superscripts is significantly ($P < 0.01$) differed.			

Table 3

Effect of *Chlorella vulgaris* or *Spirulina platensis* supplementation on seminal picture in lead acetate treated rats.

Groups	Total sperm count (x106/ml)	Total malformation sperms (%)
Control	$81.62^{a} \pm 1.14$	$10^{d} \pm 0.3$
Lead	$60.18^{d} \pm 0.61$	$40.05^{a} \pm 0.52$
Lead + C. vulgaris	$71.27^{c} \pm 0.68$	$30.98^{b} \pm 0.55$
Lead + S. platensis	$77.91^{b} \pm 0.67$	$25.36^{c} \pm 0.39$
Within the same column, means ± SE with different superscripts is significantly ($P < 0.01$) differed.		

Table 4

Effect of *Chlorella vulgaris* or *Spirulina platensis* supplementation on serum testicular enzymes activities and sex hormones levels in lead acetate treated rats.

Groups	ACP activity (U/L)	SDH activity (ng/ml)	Testosterone level (ng/ml)	FSH level (mIU/ml)	LH level (mIU/ml)
Control	$4.60^{d} \pm 0.02$	$11.13^{d} \pm 0.11$	$4.72^{a} \pm 0.03$	$3.24^{d} \pm 0.04$	$6.48^{d} \pm 0.04$
Lead	$7.83^{a} \pm 0.10$	$29.23^{a} \pm 0.28$	$2.48^{d} \pm 0.03$	$5.77^{a} \pm 0.03$	$10.96^{a} \pm 0.08$
Lead + C. vulgaris	$6.99^{b} \pm 0.04$	$22.59^{b} \pm 0.24$	$3.05^{c} \pm 0.06$	$4.92^{b} \pm 0.05$	$8.95^{b} \pm 0.06$
Lead + S. platensis	$6.08^{c} \pm 0.07$	$18.15^{c} \pm 0.15$	$3.73^{b} \pm 0.05$	$3.99^{c} \pm 0.07$	$8.10^{c} \pm 0.09$
Within the same column, means ± SE with different superscripts is significantly ($P < 0.01$) differed.					

Table 5

Effect of *Chlorella vulgaris* or *Spirulina platensis* supplementation on testicular oxidative stress, inflammatory, and apoptosis indices in lead acetate treated rats.

Groups	TAC (mmol/g tissue)	TOC (mmol/g tissue)	TNF-α level (pg/mg tissue)	Caspase-3 level (ng/mg tissue)
Control	$0.124^{a} \pm 0.002$	$0.249^{d} \pm 0.004$	$15.37^{d} \pm 0.16$	$5.29^{d} \pm 0.05$
Lead	$0.062^{d} \pm 0.003$	$0.607^{a} \pm 0.008$	$29.63^{a} \pm 0.23$	$11.29^{a} \pm 0.23$
Lead + C. vulgaris	$0.078^{c} \pm 0.002$	$0.495^{b} \pm 0.004$	$25.07^{b} \pm 0.18$	$9.08^{b} \pm 0.09$
Lead + S. platensis	$0.091^{b} \pm 0.003$	$0.411^{c} \pm 0.010$	$21.54^{c} \pm 0.33$	$8.03^{c} \pm 0.12$
Within the same column, means ± SE with different superscripts is significantly ($P < 0.01$) differed.				

Figures

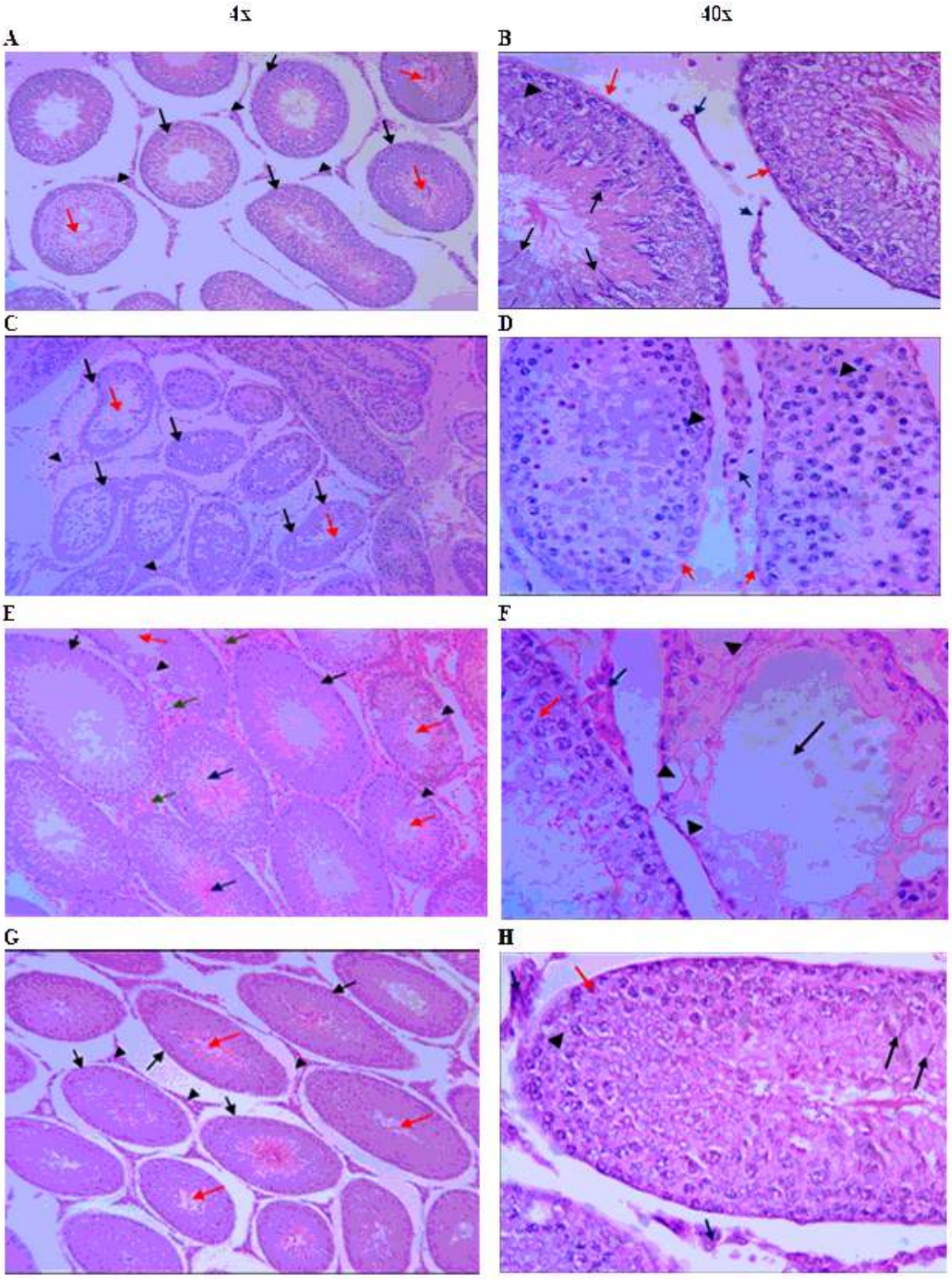

Figure 1

Graphical photomicrographs of testicular sections from rats treated with C. vulgaris or S. platensis versus lead acetate revealing (Hx&E) between the experimental groups; (A, B): control group delineated seminiferous tubules (ST) with well-defined regular basement membrane and germinal epithelium (black arrows, 4x), (Red arrows 40x). In addition to presence of regularly arranged Spermatogenic cells and uniform maturation of spermatozoa formation in ST (red arrow, 4x), (arrow head, 40x). As well as,

normally organized Leydig cells are seen (arrow heads, 4x), (blue arrows, 40x). (C,D): Lead group outlined ST suffered from thinned irregular basement membrane, apparent disarrangement and damage (black arrows 4x), (red arrows 40x). In addition to Spermatogenesis distribution with no evidence of spermatozoa formation (red arrow 4x), (arrow head 40x) and hyperplasia of Leydig cells (arrow heads 4x), (blue arrows, 40x). (E,F): Lead + C. vulgaris group exhibited ST with regular shape and outline, and closely backed (Black arrows, 4x). Spermatogenesis is maintained in some tubules (50% of tubules) (Blue arrows 4x), while others show vacuolization (arrow heads 4x, 40x) with evidence of disturbed spermatogenesis in line with absence of spermatozoa formation (red arrow 4x), (black arrow 40x). Moreover, interstetium show Leydig cell hyperplasia (green arrows 4x), (blue arrows 40x). (G,H): Lead + S.platensis group showed that Seminiferous tubules are evenly spaced, uniform in shape, with regular outlines (black arrows, 4x), (red arrows, 40x). Spermatogenic cells (arrow head, 40x) revealed uniform spermatogenesis and spermatozoa formation are evident in most of tubules (red arrow 4x), (black arrows 40x). In addition to, diminution of Leydig cells hyperplasia (arrow heads, 4x), (blue arrows, 40x).

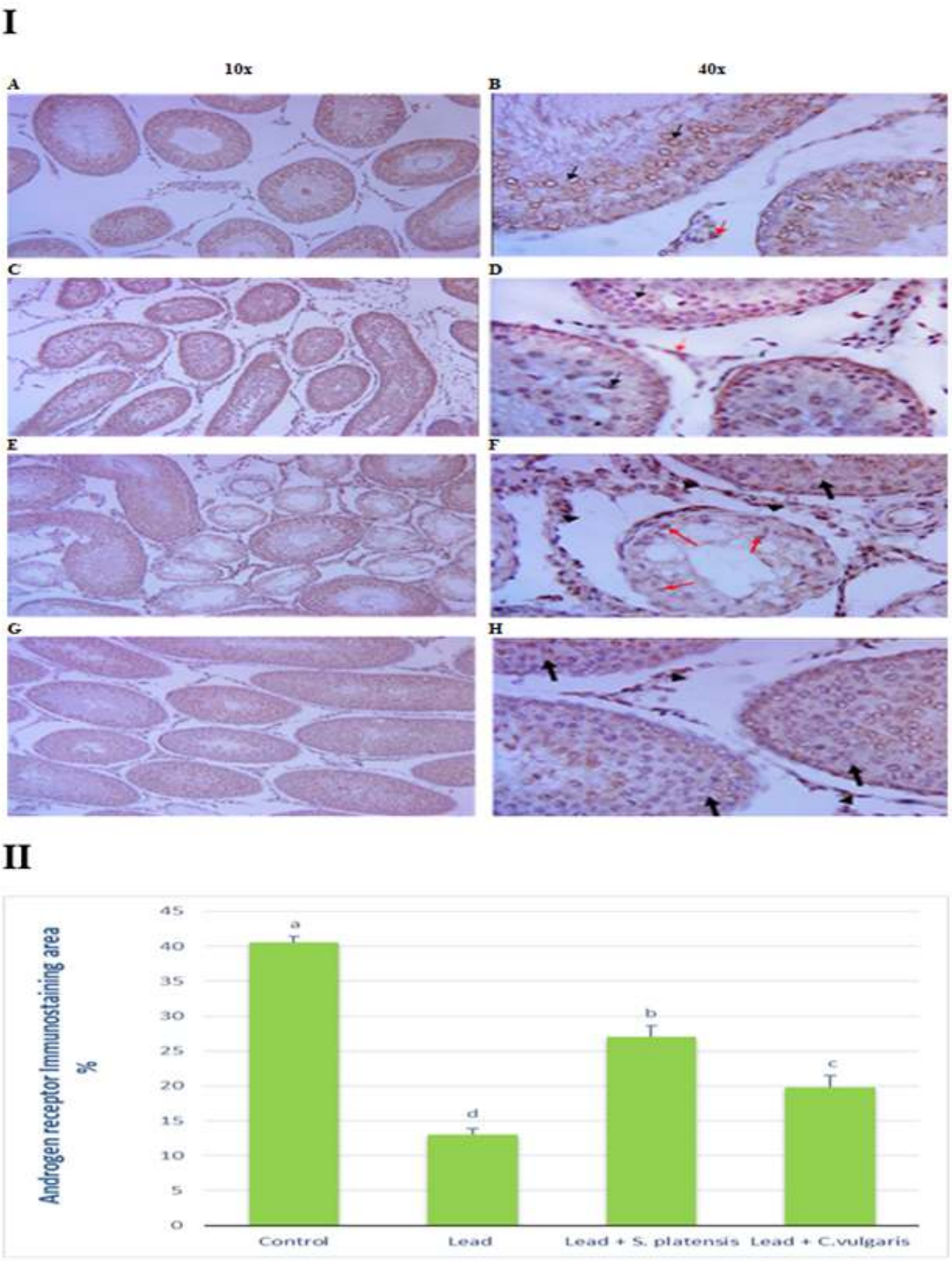

Figure 2

I. Graphical photomicrographs of testicular sections from rats treated with C. vulgaris or S. platensis versus lead acetate revealing androgen receptor Immunostaining area % between the experimental groups;(A,B): control group outlined that nuclear AR expression pattern was strong in Spermatogenic cells (black arrows, 40x) and Leydig cells (red arrow, 40x).(C,D): Lead group exhibited weak staining intensity of the expression area of AR in nuclei of Spermatogenic cells (black arrow, 40x) and moderate staining

intensity in Leydig cells (red arrow, 40x). (E,F): Lead + C. vulgaris group revealed moderate expression of AR in spermatogenic cells in uniform tubule (upper right; black arrow, 40x). While in injured tubules there is weak expression of AR in nuclei of remaining spermatogenic cells (red arrow, 40x). There is strong AR expression in Leydig cells (arrow heads, 40x). (G,H): Lead + S.platensis group delineated moderate to strong nuclear expression of AR in nuclei of Spermatogenic cell (black arrow, 40x). As well as, there is strong AR expression in Leydig cells (arrow heads, 40x). II. Column chart revealing the androgen receptor Immunostaining area % in rats treated with C. vulgaris or S. platensis versus lead acetate. Data are expressed as mean ± SE. Means with different superscripts are significantly ($P < 0.01$) differed.