

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

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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- μ m 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% H₂O₂ 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 \pm 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 3 β - and 17 β -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

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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

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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.).

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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 ± 2.60	1.62 ^a ± 0.02	1.63 ^a ± 0.02	0.43 ^a ± 0.01
Lead	66.38 ^c ± 1.71	1.23 ^c ± 0.03	1.23 ^c ± 0.02	0.34 ^c ± 0.01
Lead + <i>C. vulgaris</i>	83.00 ^b ± 1.72	1.37 ^b ± 0.03	1.37 ^b ± 0.03	0.39 ^b ± 0.01
Lead + <i>S. platensis</i>	86.25 ^b ± 1.84	1.47 ^b ± 0.05	1.45 ^b ± 0.04	0.40 ^{ab} ± 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 ± 0.03	0.94 ^a ± 0.04	0.76 ^a ± 0.03
Lead	0.22 ^d ± 0.02	0.35 ^c ± 0.01	0.32 ^c ± 0.04
Lead + <i>C. vulgaris</i>	0.34 ^c ± 0.02	0.64 ^b ± 0.04	0.57 ^b ± 0.05
Lead + <i>S. platensis</i>	0.45 ^b ± 0.04	0.79 ^a ± 0.05	0.68 ^{ab} ± 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 (x10 ⁶ /ml)	Total malformation sperms (%)
Control	81.62 ^a ± 1.14	10 ^d ± 0.3
Lead	60.18 ^d ± 0.61	40.05 ^a ± 0.52
Lead + <i>C. vulgaris</i>	71.27 ^c ± 0.68	30.98 ^b ± 0.55
Lead + <i>S. platensis</i>	77.91 ^b ± 0.67	25.36 ^c ± 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 ± 0.02	11.13 ^d ± 0.11	4.72 ^a ± 0.03	3.24 ^d ± 0.04	6.48 ^d ± 0.04
Lead	7.83 ^a ± 0.10	29.23 ^a ± 0.28	2.48 ^d ± 0.03	5.77 ^a ± 0.03	10.96 ^a ± 0.08
Lead + <i>C. vulgaris</i>	6.99 ^b ± 0.04	22.59 ^b ± 0.24	3.05 ^c ± 0.06	4.92 ^b ± 0.05	8.95 ^b ± 0.06
Lead + <i>S. platensis</i>	6.08 ^c ± 0.07	18.15 ^c ± 0.15	3.73 ^b ± 0.05	3.99 ^c ± 0.07	8.10 ^c ± 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 + <i>C. vulgaris</i>	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 + <i>S. platensis</i>	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 \pm SE with different superscripts is significantly (P < 0.01) differed.				

Figures

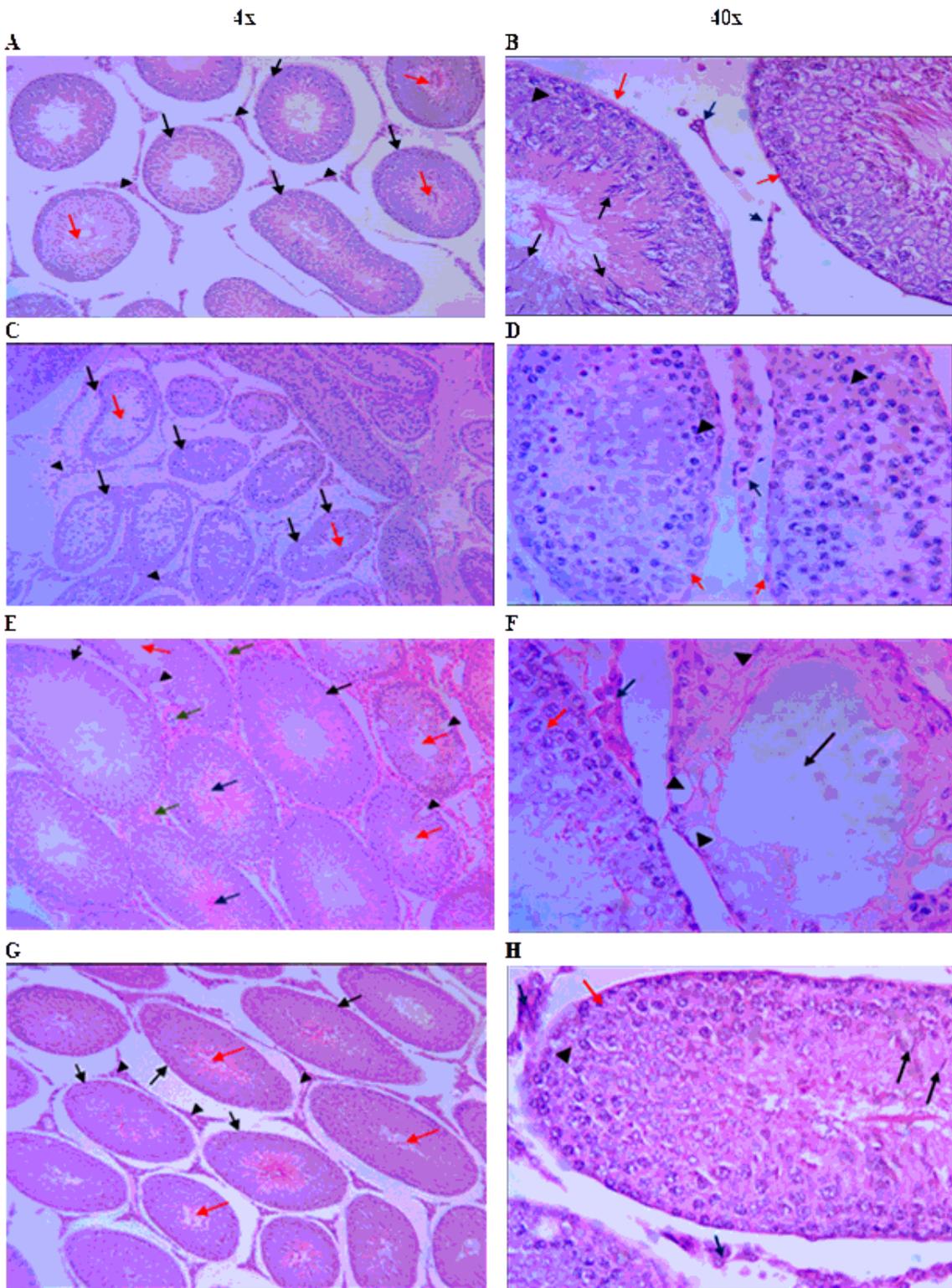
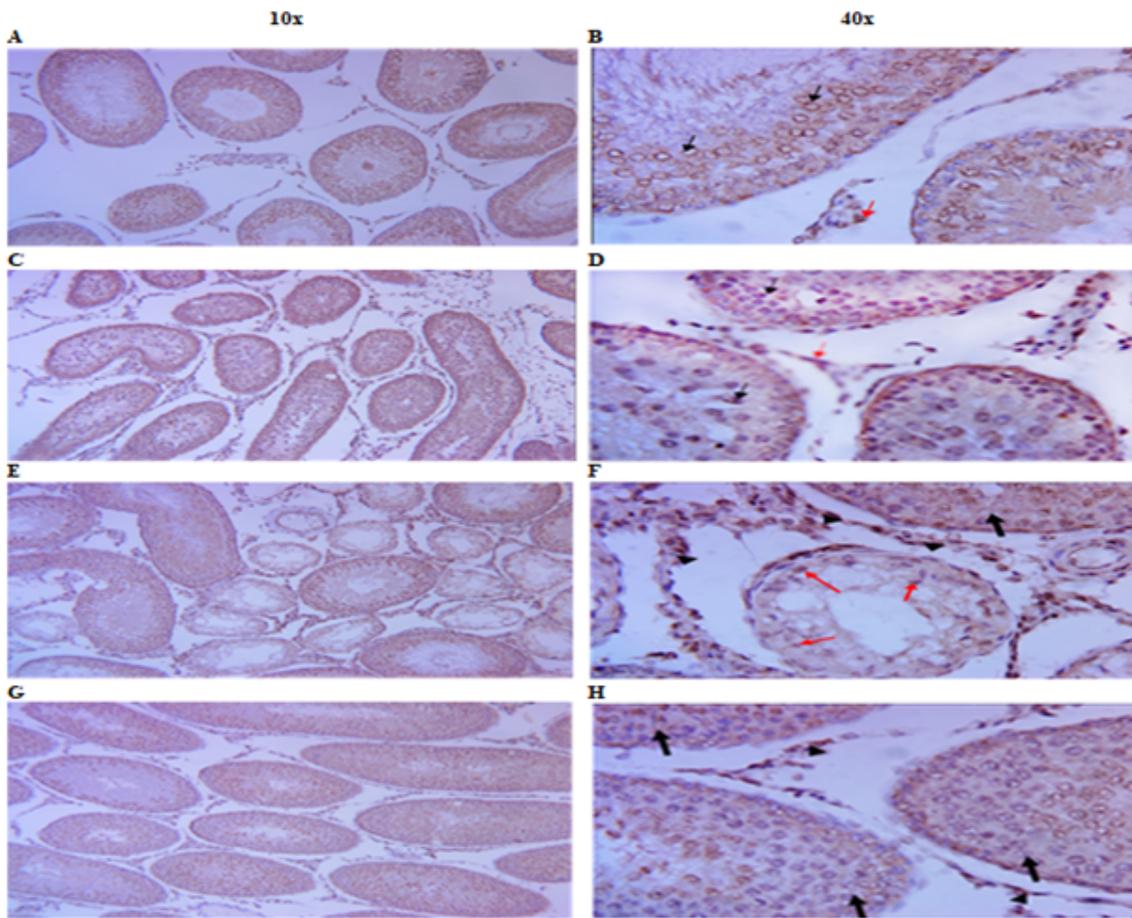


Figure 1

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).

I



II

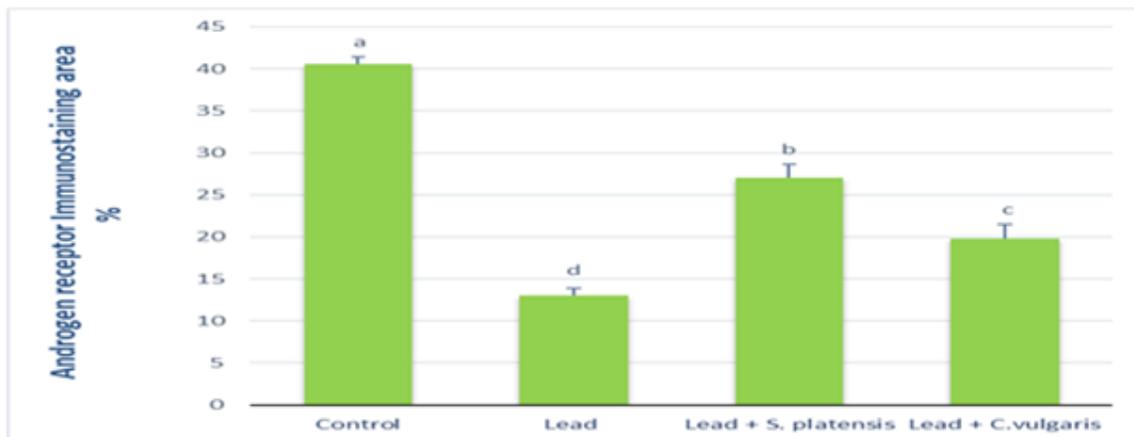


Figure 2

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 \pm SE. Means with different superscripts are significantly ($P < 0.01$) differed.