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Spirulina platensis shields the submandibular gland from cadmium toxicity by bolstering antioxidant defenses and maintaining its structural integrity

Eman Mohamed Faruk [a,b,*], Fatma Ibrahim [c], Dalia Ibrahim El-wafaey [d], Yasmeen Mohammed Ismail El Sayed [e], Mohamed Ghazy Attia Hablas [f], Mahmoud M. Hassan [g], Ahmed Mohammed Zaazaa [h], Kamal M. Kamal [f]

[a] Anatomy Department, College of Medicine, Umm Al-Qura University, Makkah, Saudi Arabia
[b] Department of Histology and Cell Biology, Faculty of Medicine, Benha University, Benha, Egypt
[c] Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Zagazig University, Zagazig, Egypt
[d] Department of Human Anatomy and Embryology, Faculty of Medicine, Zagazig University, Egypt
[e] Department of Pharmacology, Faculty of Medicine, Benha University, Benha, Egypt
[f] Department of Histology and Cell Biology, Faculty of Medicine, Suez University, Egypt
[g] Department of Physiology, Faculty of Medicine, Benha University, Benha, Egypt
[h] Student at Faculty of Medicine, Benha National University, Benha Colleges in Cairo, Egypt

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ABSTRACT

Cadmium (Cd), an element categorized as a non-essential transitional metal, has potential hazards to the health of both human beings and animals. *Spirulina platensis* (SP), a type of blue-green algae, possesses a high concentration of essential antioxidants. The present study aimed to explore the possible defensive role of SP against Cd-induced submandibular gland injury in rats by assessment of biomarkers related to both oxidative stress and inflammatory processes, which were further explored through histopathological examination of submandibular gland tissue. Consequently, the study included 32 mature rats, subdivided into four different groups as follows: control, SP, Cadmium chloride ($CdCl_2$), and $CdCl_2$/SP. The duration of the study was 24days. The results revealed that $CdCl_2$ induced submandibular gland injury as shown by the oxidant/antioxidant imbalance and increased inflammatory reactions, in addition to, histopathological changes and overexpression of BAX immunostaining. Concurrent SP administration to CdCl2-treated rats significantly improved all these effects. We concluded that concurrent SP supplement improved the submandibular gland injury provoked by $CdCl_2$.

1. Introduction

Cadmium (Cd), an element categorized as a non-essential transitional metal, possesses toxic properties and potential hazards to the health of both human beings and animals as well, it is noteworthy to mention that the toxicity of Cd remains remarkably high, even at extremely minute concentrations [1]. It is a naturally occurring environmental pollutant that is produced by agricultural and manufacturing sources [2]. Cd is largely used in several industries such as paints, metal coating, mining, batteries, and fertilizers [3].

Cadmium is toxic both by inhalation and ingestion causing acute and chronic toxicities. Some edible foodstuffs exhibit selective uptake of Cd, therefore food is frequently described as a primary route through which humans are exposed to Cd [4]. Food and tobacco smoking together are the most significant sources of Cd for human beings [5].

Cd accumulates in nearly all of the human tissues with a biological half-life that has been assessed to be more than 10 years [6]. It was evidenced that consuming water from natural sources leads to the high Cd accumulation in the renal cortex and the liver of individuals living in the industrial regions [7]. Cd in blood is an indicator of recent exposure, while Cd in urine mirrors the body burden and reveals chronic exposure [8].

The generation of reactive oxygen species (ROS) for instance superoxide anion, hydrogen peroxide, and hydroxyl radical, induced by Cd, results in the activation of signaling pathways. This, in turn, stimulates autophagy, apoptosis, and gene expression, denoting that oxidative stress is a chief mechanism of Cd toxicity [9]. Also, Cd targets the sulfhydryl groups present in proteins causing its inactivation, which can

* Corresponding author at: Faculty of Medicine, Department of Histology and Cell Biology, Benha University, 44519, Egypt.
E-mail address: faruk_eman@yahoo.com (E.M. Faruk).

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result in multiple functional defects in the nuclei, the endoplasmic reticulum, and mitochondria [10]. Cd is a mitogen and promotes cancer in several tissues. It is proven to be a human carcinogen (group I of the International Agency for Research on Cancer classification) [11].

The equilibrium between oxidant formation and endogenous antioxidant protective mechanisms is required. If this balance is disturbed, it can lead to oxidative stress-induced tissue damage [12].

Spirulina platensis (SP), a type of blue-green algae, possesses a high concentration of essential antioxidants, for instance, phycocyanin, carotenoids, phenolic compounds, microelements, vitamins, amino acids, and fatty acids besides antioxidant enzymes, such as superoxide dismutase, catalase, and peroxidase [13,14].

Spirulina platensis usage is well-established in prophylaxis besides treatment of several toxicity models, for example, acrylamide [15], fluoride [16], cyclophosphamide [17], arsenic [18], lead [19], mercury [20], manganese [21], and cadmium [22].

Considering this perspective, this study aimed to recognize the defensive properties of SP against $CdCl_2$-triggered submandibular gland impairment. As far as our knowledge extends, this is the foremost study that has been conducted to explore these effects.

2. Material and methods

2.1. Tested compounds and chemicals

Cadmium chloride ($CdCl_2$) was acquired from Sigma–Aldrich Chemicals Co., St. Louis, USA (Catalogue number: 202908). *Spirulina platensis* (SP), in the form of dark greenish fine particles, was obtained from El-hellow for biological products, Cairo, Egypt. Normal saline (0.9 % sodium chloride), used as a vehicle, was obtained from Nile Company, Cairo.

2.2. Animals and study design

The study encompassed 32 adult male albino rats, with an age range of 6–8 weeks and weight range of 180–200 gm, obtained from the Zagazig Scientific and Medical Research Center (ZSMRC), Faculty of Medicine, Zagazig University, where the study had been conducted also. The present study has been approved by the Institutional Animal Care and Use Committee (IACUC) at Zagazig University, as evidenced by the assigned approval number: ZU-IACUC/3/F/413/2022. All experimental techniques were executed in agreement with the guidelines established for the ethical treatment and use of laboratory animals.

After two weeks of acclimatization to guarantee their physical health and to exclude any sick animals, the rats were weighed and divided into equal four groups, with eight rats in each.

Group I (Control): rats were given normal saline (1 ml) that was used also as a vehicle to dissolve SP and $CdCl_2$.

Group II (SP): rats were given 300 mg/kg/b.w. of SP [23].

Group III ($CdCl_2$): rats were given 10 mg/kg/b.w. of $CdCl_2$ [24,25].

Group IV ($CdCl_2$/SP): rats were given SP (300 mg/kg/b.w.) and $CdCl_2$ (10 mg/kg/b.w.).

All treatments were given by oral gavage, once/day for successive 24 days.

2.3. Blood and submandibular tissue preparation

Upon completion of the treatment protocol, the body weight of every rat was documented to accurately assess any weight changes.

Blood samples were collected using clean test tubes and then centrifuged for 5 min for separation of the serum. The supernatant sera were used to measure malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH), and total antioxidant capacity (TAC). Then, all rats were scarified via cervical dislocation, and the submandibular glands were dissected and weighed. The submandibular glands were separated into two parts. The first one was homogenized for measurement of transforming growth factor-beta (TGF-β), tumor necrosis factor-alpha (TNF-α), Interleukin 1-beta (IL-1β), and Interleukin-10 (IL-10). The other part was prepared for histological studies.

2.4. Determination of the activity of oxidant and antioxidant biomarkers

The levels of MDA (CAT. No. MD 25 29), CAT (CAT. No. CA 25 17), SOD (CAT. No. SD 25 21), GSH (CAT. No. GR 25 11) and TAC (CAT. No. TA 25 13) were measured by the specific kits purchased from Biodiagnostic, Egypt.

2.5. Determination of inflammatory markers in submandibular tissue

Based on enzyme-linked immuno-sorbent assay (ELISA), the levels of TGF-β, TNF-α, IL-1β, and IL-10 were assessed by the commercial ELISA kits purchased from (eBioscience, Inc., San Diego, CA, USA) as stated by manufacturer's guidelines.

2.6. Histological study

2.6.1. Light microscopy

The submandibular glands tissues were dissected and fixed in 10 % formalin for processing of paraffin sections. 5 μm-thickness sections that were prepared and processed for H&E staining and PAS staining (for detection of mucopolysaccharides) [26] then examined by a light microscope. We performed comprehensive histological analyses for regional differences in the acini, myoepithelium, ducts, blood vessels, and the extracellular matrix (ECM) surrounding acini and ducts. Serous acini, showing abundant basophilic cytoplasm with a round nucleus, and mucous acini with slightly basophilic cytoplasm.

2.6.2. Immunohistochemistry

For the immunohistochemistry, the method of peroxidase-labelled streptavidin–biotin was employed [27]. It was done to detect the Bax proteins. The antibodies employed in the detection of Bax consisted of rabbit polyclonal antibodies obtained from Sigma-Aldrich, Egypt, and were utilized at a 1:50 dilution rate. The specific Catalog No. for the antibodies was SAB4502549. The reaction was shown as brownish cytoplasmic discoloration. For negative control sections, the primary antibodies were excluded. Positive control of BAX was taken from a human breast cancer specimen.

2.6.3. Histomorphometric analysis

The morphometric analysis of the sections was conducted utilizing the Leica Qwin 500 Image Analyzer Computer System, located at the Pathology Department of the Faculty of Medicine at Cairo University. To evaluate the mean area percentage of PAS staining and Bax immunostaining among the studied groups, ten distinct fields were evaluated in each slide, at lower magnification. The histological parameters were semi-quantitatively defined by essential histological features, including the degree of fatty replacement, preservation of lobular architecture, preservation of ducts and acini, the presence of interstitial fibrosis, and the inflammatory component. The scales used were no degeneration (0), trace (1), mild (2), moderate (3), and severe (4) degeneration.

The non-parametric Cuzick's test for trend [28] was used to compare the grades of fatty replacement, loss of lobular architecture, presence of interstitial fibrosis, degree of ductal degeneration, presence of diffuse inflammatory component, presence of focal inflammatory component, and degree of acinar degeneration across these four groups.

Each parameter was scored (1–4 points), so the total point score for each rat was ranging between 6 and 24. The obtained scores were divided by six for interpretation. Less than 1: no degeneration, 1.0–2.5: mild degeneration, and 2.6–4.0: severe degeneration.

2.7. Statistical analysis

The variables were presented in the form of mean ± standard deviation (SD) and were subjected to comparison through the application of one-way analysis of variance (ANOVA) and post-Hoc Tukey test to determine the differences between groups. Differences were considered significant when the *P*-value was less than 0.05. IBM SPSS software was used for statistical analysis.

3. Results

3.1. SP effectively attenuated $CdCl_2$ toxicity affecting mortality rates, body weight, and the weight of the submandibular gland

There were no recorded fatalities within any of the studied groups. $CdCl_2$ treatment for successive 24 days resulted in a statistically significant body weight loss compared with the other studied groups ($P < 0.05$). Regarding the submandibular gland weight, there was no significant difference in the $CdCl_2$/SP when compared to the control and SP groups ($P > .05$) (Table 1).

3.2. SP remarkably restored $CdCl_2$-induced oxidative stress and inflammatory biomarkers imbalance in the rats

This study revealed that there were no statistically significant differences in mean values of all studied biochemical parameters in the SP group when compared with the control group ($P > .05$).

$CdCl_2$-intoxicated rats showed increased oxidative stress represented by statistically significantly higher MDA levels and lower catalase, SOD, GSH, and TAC activities compared with the other studied groups ($P < 0.05$). Concurrent SP administration to $CdCl_2$-intoxicated rats induced a significant decrease in the MDA level and an increase in catalase, SOD, GSH, and TAC activities (Table 2).

The results of Table 3 showed that $CdCl_2$ significantly increased TGF-β, TNF-α, and IL-1β levels along with significantly decreased IL-10 levels. The SP administration had protected against these alterations represented by a significant lowering of the levels of TGF-β, TNF-α, and IL-1β and a significant elevation of IL-10 level.

3.3. SP alleviated $CdCl_2$-induced submandibular histopathological and histochemical alternation in the rats

Histological analysis of H&E-stained sections of the submandibular salivary gland in the control and SP groups showed normal parenchyma with many lobules that contain both pale vacuolated mucous acini with flat nuclei and serous acini (with rounded nuclei and darkly stained secretory granules) and serous demilunes (capped acini). Ducts were found between acini in which intralobular, and the intercalated ducts were characterized by their simple cuboidal epithelium lining, while the striated ducts were distinguished by their lining of high cuboidal cells.

Table 1
Effects of cadmium chloride and *Spirulina platensis* administrations on the initial body weight, final body weight, and submandibular gland weight.

Parameter	Groups			
	Control	SP	$CdCl_2$	$CdCl_2$/SP
Initial body weight (gm)	185.38 ± 5.10	187.38 ± 3.29	185.50 ± 3.67	184.63 ± 2.67
Final body weight (gm)	239.13 ± 3.64	241.38 ± 6.48	203.25 ± 4.89 ^{ab}	224.75 ± 3.06^{abc}
Submandibular weight (gm)	0.43 ± 0.02	0.42 ± 0.01	0.35 ± 0.03 ^{ab}	0.40 ± 0.04^{c}

Each value is mean ± SD for eight rats in each group.
[a]: $P < 0.05$ compared to control, [b]: $P < 0.05$ compared to SP, [c]: $P < 0.05$ compared to $CdCl_2$.
Abbreviations; SP, *Spirulina platensis*; $CdCl_2$, Cadmium chloride.

Table 2
Effects of cadmium chloride and *Spirulina platensis* administrations on the oxidant and antioxidant biomarkers.

Parameter	Groups			
	Control	SP	$CdCl_2$	$CdCl_2$/SP
Serum MDA (nmol /ml)	1.55 ± 0.30	1.52 ± 0.32	3.93 ± 0.41 ^{ab}	2.97 ± 0.12 ^{abc}
Serum Catalase (U/L)	129.03 ± 2.20	127.62 ± 3.56	66.91 ± 4.11^{ab}	99.29 ± 3.19^{abc}
Serum SOD (U/ml)	371.73 ± 9.29	368.19 ± 3.52	95.70 ± 6.72^{ab}	176.04 ± 6.80^{abc}
Serum GSH (mmol/L)	5.08 ± 0.12	5.02 ± 0.15	1.48 ± 0.18^{ab}	3.52 ± 0.18^{abc}
Serum TAC (mmol /L)	1.88 ± 0.12	1.84 ± 0.11	0.64 ± 0.04 ^{ab}	0.93 ± 0.06 ^{abc}

Each value is mean ± SD for eight rats in each group.
[a]: $P < 0.05$ compared to control, [b]: $P < 0.05$ compared to SP, [c]: $P < 0.05$ compared to $CdCl_2$.
Abbreviations; MDA, Malondialdehyde; SOD, Superoxide dismutase; GSH, Reduced glutathione; TAC, Total antioxidant capacity; SP, *Spirulina platensis*; $CdCl_2$, Cadmium chloride.

Table 3
Effects of cadmium chloride and *Spirulina platensis* administrations on the pro-inflammatory and fibrotic markers.

Parameter	Groups			
	Control	SP	$CdCl_2$	$CdCl_2$/SP
Tissue TGF-β pg/100 mg tissue)	175.75 ± 4.81	177.43 ± 1.63	412.87 ± 5.24 ^{ab}	330.88 ± 5.27 ^{abc}
Tissue TNF-α (pg/ 100 mg tissue)	20.94 ± 1.26	21.13 ± 1.82	54.51 ± 4.61 ^{ab}	37.75 ± 2.93 ^{abc}
Tissue IL-10 (pg/100 mg tissue)	377.22 ± 6.63	372.03 ± 5.24	133.01 ± 4.60^{ab}	234.22 ± 4.62^{abc}
Tissue IL-1β (pg/100 mg tissue)	86.54 ± 1.06	88.08 ± 1.09	314.02 ± 2.54 ^{ab}	250.43 ± 5.01 ^{abc}

Each value is mean ± SD for eight rats in each group.
[a]: $P < 0.05$ compared to control, [b]: $P < 0.05$ compared to SP, [c]: $P < 0.05$ compared to $CdCl_2$.
Abbreviations; TGF-β, Transforming growth factor-beta; TNF–α, Tumor necrosis factor–alpha; IL- IL-10, Interleukin-10; IL-1β, Interleukin-1β; SP, *Spirulina platensis*; $CdCl_2$, Cadmium chloride.

The lobes of the gland were separated by connective tissue septa. (Fig. 1A and 1B).

Stained sections by H&E of the submandibular gland from the $CdCl_2$ group showed massive histological changes in the acini, as they degenerated, disarranged with disturbance of their architecture, showing widely cellular detached by connective tissue that contained dilated congested blood vessels (Fig. 1C and 1D). Some intralobular ducts displayed disarrangement of the lining epithelial cells with hyperchromatic nuclei and fatty infiltration (Fig. 1E and 1F). While the $CdCl_2$/SP group showed normal acinar structure with the normal arrangement of acini and ducts with increasing in their number (Fig. 1G).

Histochemical examination of PAS-stained sections of the submandibular salivary gland in the control and SP groups showed that most of the acinar cells contain a mixture of both neutral and acid polysaccharides as they have deep purple color, while the duct system showed faint PAS-positive reaction (Fig. 2A and 2B).

Stained sections of the submandibular gland by PAS from the $CdCl_2$ group showed a marked decrease in the number of neutral mucopolysaccharides as there was a decrease in the purple staining in comparison to that observed in control sections (Fig. 2C). The $CdCl_2$/SP group revealed normal PAS + ve acinar cells due to an increase in the content of mucopolysaccharides (Fig. 2D).