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Influence of *Spirulina platensis* exudates on the endocrine and nervous systems of a mammalian model

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ABSTRACT

Objective: To investigate the effect of intra-peritoneal injection of purified exudates of axenic *Spirulina platensis* on the mammalian endocrine and nervous systems.

Methods: The intra-peritoneal injection of the cyanobacterial exudates in mice was applied. Sex hormonal levels of testosterone and progesterone were measured using radioimmunoassay while the follicular stimulating hormone and luteinizing hormone were evaluated by direct chemiluminescence. In addition, superoxide dismutase, catalase and glutathione peroxidase were monitored in the hippocampus region using spectrophotometric method. The levels of the hippocampal monoamines, dopamine, noradrenaline and serotonin were analyzed by high performance liquid chromatography while the acetyl choline neurotransmitter was measured by colorimetric method using choline/acetylcholine assay kit.

Results: A sharp disruption in the sex hormones levels of testosterone, progesterone, follicular stimulating hormone and luteinizing hormone was demonstrated in the serum of the treated mice. At the same time, a significant reduction in the endogenous antioxidant defense enzymes, superoxide dismutase, catalase and glutathione peroxidase was observed in the hippocampus region of the injected mice. Moreover, levels of dopamine, noradrenaline, serotonin and acetyl choline neurotransmitter in the same region were significantly affected as a result of the treatment with *Spirulina* filtrate. The gas chromatography-mass spectrometer and liquid chromatography mass spectrometry/mass spectrometry analysis showed the presence of some sterol-like compounds in the cyanobacterial filtrate.

Conclusions: This study demonstrated the capability of *Spirulina* to release detrimental bioactive metabolites into their surrounding that can disrupt the mammalian endocrine and nervous systems.

1. Introduction

Spirulina is a filamentous, spiral-shaped, multicellular and photosynthetic cyanobacteria. It is dominating the microflora of alkaline saline waters with pH up to 11.0 and can be found in diverse kinds of environments such as soils, fresh, brackish, seawaters as well as industrial and domestic wastewater. *Spirulina* contains up to 70% complete protein that contains all the essential amino acids[1]. It is also rich in essential fatty acids, vitamins, minerals, photosynthetic pigments, polysaccharides, glycolipids and sulfolipids[2]. This cyanobacterium is cultivated around the world and is used as a primary human dietary supplement[3,4]. It has been stated by National Aeronautics and Space Administration (NASA) that the nutritional value of one kilogram of *Spirulina* is equivalent to one thousand kilogram of fruits and vegetables. Therefore in long-term space missions, NASA proposed that *Spirulina* served as a major source of food and nutrition[5]. It is also used as a feed supplement in the aquaculture and poultry industries[6,7].

In addition to the nutritional advantages, *Spirulina* has other

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beneficial characteristics such as antibacterial, antifungal, antiviral, molluscicidal, anticancer, anti-inflammatory and antioxidant activities as well as its stimulant effects on innate and specific immunity[8-13]. Moreover, *Spirulina* was used successfully for the clinical improvement of anaemia in children and the protection against hay fever[14,15].

On the other hand, minor contraindications of using *Spirulina* as a human dietary supplement including headache, muscle pain, sweating, difficulty concentrating, and skin reactions were recorded[16]. At the same time, *Spirulina* may contain the hepatotoxin, microcystin, which is accumulated in the liver and can potentially cause cancer or other liver diseases[17]. Furthermore, Jiang *et al.* reported the existence of microcystins in 36 kinds of cyanobacteria *Spirulina* health food samples obtained from various retail outlets in China[18]. About 94% of the samples contained low concentrations of microcystins. They concluded that the possible potential health risks from chronic exposure to these toxins should not be ignored, even if the toxin concentration was low.

Another harmful effect of *Spirulina platensis* (*S. platensis*) was recorded by Mazokopakis *et al.* who correlated the ingestion of *Arthrospira platensis* with a human life threaten disease called rhabdomyolysis[19]. This disease leads to release of muscle cell contents into the systemic circulation. Symptoms of acute rhabdomyolysis including muscular weakness, swelling, pain, cramping and tea-colored urine were recorded with a patient taking *Arthrospira platensis* as a dietary supplement.

It has been reported that certain cyanobacterial species can produce the neurotoxic amino acid β-N-methylamino-L-alanine (BMAA) [20,21]. The exposure to such these metabolites might be involved in the etiology of certain neurodegenerative diseases[22]. Subsequent testing of a variety of cyanobacterial species revealed that over 90% of the tested genera could produce BMAA[21,23]. Additionally, it has been recorded that under certain conditions, *Spirulina*, seems to be able to produce some metabolites that can induce a neurological disease (amyotrophic lateral sclerosis-parkinsonism-dementia complex)[24].

In our previous study[25], it was shown that the intra-peritoneal injection of some cyanobacterial exudates in mice demonstrated a remarkable disruption of serum sex hormonal levels. Hence, the aim of this study is to investigate the effect of the intra-peritoneal injection of *S. platensis* exudates on the serum sex hormones as well as the antioxidant enzymes and the neurotransmitters in the hippocampus region of the treated mice.

2. Materials and methods

2.1. Ethics statement

This study was carried out in strict compliance with the Guidelines of Animals Health Research Institute, Egypt. The study protocol was approved by the Committee on the Ethics of Animals Health Research Institute, Egypt (Permit Number 362 approved on August 31, 2010). Mice were sacrificed by decapitation after narcotizing with aerosolized isoflurane. Regarding water samples collection from lakes of Wadi El-Natrun, Egypt, there were no specific permissions required and the field studies did not involve endangered or protected species.

2.2. Isolation and growth of S. platensis

Water samples were collected in 2010 from lakes of Wadi El-Natrun, Egypt (no specific permissions were required). Samples were filtered through sterilized Whatman No. 41 filter paper and then suspended on 5 mL sterilized Zarrouk medium[26]. One to two drops of each suspension were inoculated on solid Zarrouk medium and incubated for about two weeks in culture room at (25 ± 1) ℃ under controlled continuous illumination of 40 $\mu Em^{-2}s^{-1}$. The plates were examined and the best colonies were selected, picked up and re-streaked to new agar plates. Restreaking and subculturing were repeated several times to obtain unialgal cultures. To get axenic culture, the tested cyanobacterium was grown in liquid cultures for 12 d to attain vigorous growth. About 20 mL of each culture was centrifuged at 6000 r/min for 10 min and the algal pellets were then streaked on peptone or yeast extract solid medium. Those which proved not to be axenic streaks have been repeated till they become axenic. Inocula from axenic cultures were taken into sterilized liquid medium to be ready for the desired experiments. The purified cyanobacterium was identified as *S. platensis*[27].

Batch culture of *S. platensis* was grown on Zarrouk medium for 28 d under the previous conditions and cells were separated from their culture by filtration using Whatman No. 41 filter paper. The cyanobacterial biomass was subjected to chlorophyll "a" analysis while the culture filtrate was stored at 4°C. Chlorophyll "a" content in the biomass was extracted using the standard acetone extraction method[28]. After extraction, chlorophyll "a" was determined spectrophotometrically at 750 and 665 nm using a PYE Nnicom, SP8-100 UV spectrophotometer.

2.3. Purification of S. platensis exudates

The culture filtrate from the previous step was passed through glass fiber filters and 0.45 mm Millipore membranes. The filtrate was then concentrated in a rotary evaporator at 40 ℃ under reduced pressure, dialyzed successively against running tap water and distilled water in a spectra/por dialysis tube (Spectra/Por; Spectrum Medical Industries, Los Angeles, CA) with a molecular weight cut-off of 3500 daltons, and freeze dried. Weighed portions of lyophilized staff were suspended in deionized water and dispersed by storage for 18 h at 4 ℃. After that, the solution was purified using C18 solid-phase extraction discs (Empore, 3M, Minneapolis, MN, USA). Prior to extraction, the discs were conditioned with methanol and water. The *Spirulina* filtrate was extracted with 47 mm discs by applying vacuum to maintain a flow rate of 0.5 to 5 mL/min. The C18 discs were eluted three times with 5 mL of methanol each time which was blown down to dryness and stored frozen. The content was dissolved in physiological saline solution (1 mg/mL), sterilized by filtration

through a 0.22 μm filter and used for intra-peritoneal injection of the mice. Another set of the purified exudates was dissolved in methanol/water (65/35, v/v) and used for the gas chromatography-mass spectrometer (GC-MS) and liquid chromatography–mass spectrometry (LC-MS)/MS analysis.

2.4. Experimental animals

Fifty male albino mice weighing 20-25 g were housed in groups of five in polyethylene cages in a temperature controlled room under 12/12 h light/dark cycle and had free access to food pellets and tap water *ad libitum*. The minimal number of animals needed to obtain statistical significance was used and all efforts were made to minimize animal suffering. On the day of experiment, mice were divided into two groups, one group received 5 intra-peritoneal injections of 15 mg/kg of the purified *S. platensis* exudates in saline at 24 h intervals (total dose: 75 mg/kg) while the control set was given equal dose of physiological saline solution (0.85% NaCl).

2.5. Blood collection and sample preparation

One day post injection, mice were briefly anesthetized with aerosolized isoflurane and decapitated. The collected blood samples were placed at room temperature for approximately 30 min. Then, the tubes were centrifuged at 5 000 r/min for 10 min and the supernatant was collected for the assaying of sex hormones. Fresh brain samples were obtained and partitioned using a dissecting microscope. Hippocampal samples were weighed and homogenized to give 10% (w/v) homogenate in 100 mL of ice-cold dissolution buffer [0.1 mol/L perchloric acid, 0.1 mmol/L sodium bisulfite, and 0.1 mmol/L ethylene diamine tetraacetic acid (EDTA)]. All homogenates were centrifuged at 15 000 r/min for 25 min at 4 ℃. Supernatants were filtered through 0.22 mm pore size polyvinylidene fluoride membrane filters (Millipore Corp., Bedford, MA, USA) and immediately frozen and stored at -80 ℃ for the analysis of the monoamines.

Another set of the hippocampal tissues were weighed and homogenized to give 10% (w/v) homogenate in ice cold medium containing 50 mmol/L Tris-HCl and 300 mmol/L sucrose at pH 7.4. The homogenate was centrifuged at 15 000 r/min for 25 min at 4 ℃. The supernatant was filtered through polyvinylidene fluoride membrane filters and stored at -80 ℃ for the analysis of the antioxidant enzymes.

2.6. Assay of sex hormones

Serum concentrations of total testosterone, free testosterone and progesterone were measured by radioimmunoassay using a commercial kit obtained from BioSource (Nivelles, Belgium). In addition, luteinizing hormone and follicle stimulating hormone (FSH) were measured by direct chemiluminescence (ADVIA Centaur, Bayer Co., Bayer AG, Leverkusen, Germany).

2.7. High-pressure liquid chromatography (HPLC) with electrochemical detection analysis of the monoamines

The monoamines of hippocampal homogenates were analyzed using HPLC with electrochemical detection. The analysis was performed with an ESA Model 5600A Coul Array® system (ESA Inc., Chemlsford, MA, USA), equipped with Shimadzu Model DGU-14A on-line degassing unit (Shimadzu Scientific Instruments, Columbia, MD, USA), an ESA Model 582 pump and an ESA Model 542 refrigerated autosampler. Electrode potentials were selected over the range of 0 to +700 mV, with a 50 mV increment against palladium electrodes. Chromatographic separation was achieved by auto-injecting 30 μL sample aliquots at 5 ℃ onto a MetaChem Intersil (MetaChem Technologies, Torrance, CA, USA) reversed-phase C18 column with an ESA Hypersil C18 precolumn. A mobile phase flow rate of 1.25 mL/min and analysis time of 45 min were used for all experiments. System control and data processing were performed using ESA CoulArray software (version 1.02). The mobile phase containing 75 mmol/L monobasic sodium dihydrogen phosphate, 2 mmol/L sodium octylsulphonate, 25 mmol/L EDTA and 100 mL of triethylamine was prepared in 1 800 mL of HPLC grade water. This solution was then mixed with 200 mL of HPLC grade acetonitrile and buffered to pH 3.0 using concentrated ortho-phosphoric acid. Then, the mobile phase was filtered through a 0.20 mm pore size white nylon filter membrane (Millipore Corp.) and degassed under vacuum for 30 min prior to use. Stock standard solutions were prepared by dissolving 10 mg of dopamine (DA), serotonin (5-HT) and noradrenaline (NA) in 25 mL of dissolution buffer. These concentrates were then divided into 1 mL aliquots, frozen, stored at -80 ℃ and thawed prior to use at 4 ℃. All samples were processed in technical triplicate with median values used for mean calculations.

2.8. Determination of antioxidant enzymes activity

The hippocampal supernatant was used for the biochemical analysis of the antioxidant enzymes. Superoxide dismutase (SOD) activity was determined using Biodiagnostic Kit No. SD 25 21 (Biodiagnostic Co., Cairo, Egypt) which is based on the spectrophotometric method[29]. This assay relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitro blue tetrazolium dye and the absorbance was measured at 560 nm. Catalase (CAT) activity was measured using Biodiagnostic Kit No. CA 25 17 (Biodiagnostic Co., Cairo, Egypt) which is based on the spectrophotometric method[30]. CAT reacts with a known quantity of hydrogen peroxide and the reaction is stopped after 1 min with CAT inhibitor. In the presence of peroxidase, the remaining hydrogen peroxide reacts with 3,5-dichloro-2-hydroxybenzene sulfonic acid and 4-aminophenazone to form a chromophore with a color intensity inversely proportional to the amount of CAT in the sample. The absorbance was measured at 510 nm. Glutathione peroxidase (GPx) activity, which catalyzes the oxidation of reduced glutathione to glutathione disulfide, was assayed spectrophotometrically[31]. The reaction mixture consisted of 240 mIU/mL of glutathione disulfide

reductase, 1 mmol/L glutathione, 0.15 mmol/L nicotinamide adenine dinucleotide phosphate in 0.1 mol/L potassium phosphate buffer, pH 7.0, containing 1 mmol/L EDTA and 1 mmol/L sodium azide; a 50 μL sample was added to this mixture and allowed to equilibrate at 37 ˚C for 3 min and the absorbance was measured at 340 nm.

2.9. Determination of acetylcholine (Ach)

Ach level was measured by colorimetric method using choline/Ach assay kit purchased from Biovision Research Products Co., Linda Vista Avenue, Mountain View, CA, USA[32].

2.10. Analysis of sterol-like compounds

2.10.1. GC-MS analysis

GC-MS analysis was carried out in Faculty of Science (Fayoum University) on an Agilent 5973 single quadrupole mass spectrometer (Palo Alto, CA, USA), coupled with an Agilent 6890 gas chromatograph used with an Agilent DB-5.625 (30 mm × 0.25 mm inner diameter × 0.25 μm) analytical column (inlet temperature: 275 ˚C; injection volume: 2 μL; J&W Scientific, Folsom, CA, USA). The total GC run time was 53 min, and the carrier gas was helium. The initial oven temperature was held at 80 ˚C for 2 min and then increased by 10 ˚C/min till it reached 290 ˚C, after which it was held at this temperature for 30 min. The injector temperature was 290 ˚C and the split ratio was 1:30. National Institute of Standards and Technology mass spectral library was used to identify the compounds in the extracts using hexane as a control. The closest match with the highest probability in the library was recorded.

2.10.2. LC tandem MS analysis

The used MS system was a Quattro Micro mass spectrometer (Waters, Milford, MA, USA). High purity nitrogen was used as the drying and electrospray ionization (ESI) nebulizing gas and was set at 100 and 500 L/h, respectively. Argon was used as the collision gas with a gas cell pirani pressure of 3.87e-3 mbar. The capillary voltage was set at 3.5 kV and the source block and desolvation temperatures were 100 and 300 ˚C, respectively. Chromatography was performed using a Waters Alliance 2695 (Waters, Milford, MA). The column was a Water Symmetry® C18, 2.1 mm × 50 mm, 3.5 um particle size, with a guard column. The flow rate was 0.3 mL/min and an injection volume of 10 uL was used. The run time of the electrospray positive ionization (ESI^+) mode was 60 min and the mobile phase consisted of methanol/water (65/35, v/v) containing 0.3% formic acid.

2.11. Statistical methods

Results are presented as mean ± standard error of the mean. Data were compared for significant differences using Statistica Program (version 5) and student's *t*-test. The levels of significance chosen were $P < 0.05$ and $P < 0.01$.

3. Results

3.1. Effect of S. platensis exudates on the level of sex hormones

Data shown in Table 1 clarified a sharp change in the serum level of sex hormones of the male mice that were intra-peritoneally injected with the *Spirulina* exudates. A massive reduction in the total, free testosterone and progesterone was recorded (75.7%, 72.9% and 32.9%, respectively) compared with the untreated mice. On the other hand, the levels of FSH and LH were significantly elevated in the treated mice (30.9%, 40.1%).

Table 1

Levels of testosterone, progesterone, FSH and luteinizing hormone in the serum of male mice that were intra-peritoneally injected with the exudates of *S. platensis* compared with the control treated with saline solution.

Sex hormones (ng/mL)	Saline solution 0.85 g/L NaCl	Exudates of *S. platensis*
Total testosterone	48.34 ± 2.76	11.76 ± 1.43
Free testosterone	11.05 ± 1.56	2.99 ± 1.08
Progesterone	1.91 ± 0.39	1.28 ± 0.41
FSH	3.98 ± 0.48	5.21 ± 0.94
LH	1.05 ± 0.26	1.47 ± 0.32

3.2. Effect of S. platensis exudates on the hippocampal activity of antioxidant enzymes

Data in Table 2 indicated that the injection of *Spirulina* exudates led to a sharp decrease in SOD activity of the hippocampus by 42.9%. Similarly, reduced activities of CAT (8.5%) and GPx (24.9%) were detected in the hippocampus of the treated mice compared to the untreated mice.

Table 2

Effect of *S. platensis* exudates on the activity of the endogenous antioxidant enzymes in the hippocampal region of the treated mice.

Treatment	Endogenous antioxidant enzymes (μmol/min/10 mg)		
	SOD	CAT	GPx
Saline solution (0.85 g/L)	10.66 ± 1.48	33.25 ± 2.86	8.15 ± 0.91
Exudates of *S. platensis*	6.08 ± 0.97	30.41 ± 1.83	6.12 ± 1.02

3.3. Effect of S. platensis exudates on the hippocampal level of neurotransmitters

The collected data (Table 3) demonstrated that the injection of mice with *Spirulina* exudates induced variable changes in the level of monoamine neurotransmitters in the hippocampus. A significant decline in 5-HT (21.7%) was obtained, while DA and NA were increased by 16.4% and 9.5% respectively. Meanwhile, a significant reduction of the Ach concentration was recorded in the hippocampus of the treated group (25.1%).

Table 3

Effect of *S. platensis* exudates on the level of the neurotransmitters in the hippocampal region of the treated mice.

Treatment	Neurotransmitters (ng/10 mg)			
	DA	NA	5-HT	Ach
Saline solution (0.85 g/L)	31.25 ± 2.17	0.63 ± 0.42	151.05 ± 3.91	5.46 ± 1.73
Exudates of *S. platensis*	36.37 ± 1.95	0.69 ± 0.35	118.34 ± 2.94	4.09 ± 0.68

3.4. Identification of sterol-like hormones

The GC-MS analysis of the purified filtrates of *S. platensis* showed the presence of sterol-like compounds (Table 4). The identity of most peaks was determined by direct comparison to National Institute of Standards and Technology GC-MS chemical library. Compounds with retention times (17.13 min, 16.32 min, 15.95 min and 13.67 min) showed the highest similarity with corticosterone (52.1%), 2-methoxyestrone (69.8%), hydrocortisone (74.6%) and androsterone (55.5%), respectively. Due to the low similarity percentage between steroidal compounds of the *S. platensis* exudates and the available eukaryotic steroids, LC-MS/MS was used with cholesterol as reference[25]. Cholesterol molecule was broken down into product ions (88.30, 106.22 and 256.34 g/mol); the later was considered as a nucleus for the sterol-like molecules. Consequently, certain sterol-like molecules (Figure 1) with molecular mass (328.1, 354.7, 504.3 and 859.8 g/mol) were identified in the exudates of *S. platensis* at retention time 1.09 min, 2.08 min, 2.32 min and 2.78 min, respectively.

Table 4

Bioactive compounds identified from the culture filtrate of *S. platensis* revealed by GC-MS.

Retention time (min)	Molar mass (g/mol)	Close match	Similarity (%)
17.13	358	Corticosterone	52.1
16.32	356	2-methoxyestrone	69.8
15.95	316	Hydrocortisone	74.6
13.67	302	Androsterone	55.5

Data are representative of at least three independent biological replicates.

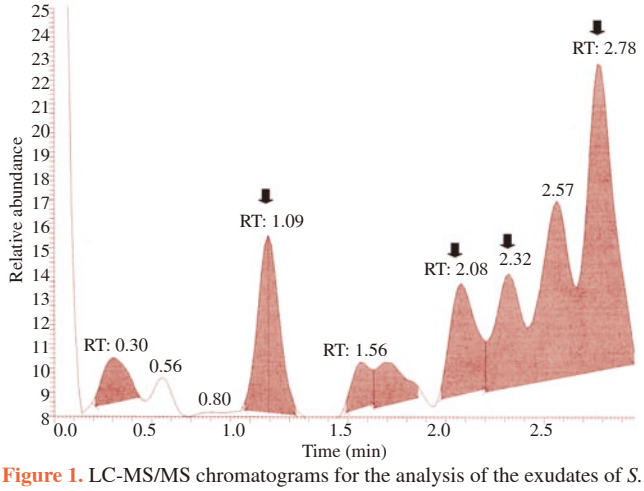

Figure 1. LC-MS/MS chromatograms for the analysis of the exudates of *S. platensis*.

Black arrows indicate the beaks that contain the sterol-like nucleus (256.32 g/mol).

4. Discussion

Although *Spirulina* possessed various positive nutritional and therapeutic properties, few studies reported its side effects. The current study clarified a sharp disturbance in the level of the sex hormones, testosterone, progesterone, FSH and LH of male mice that were intra-peritoneally injected with the *Spirulina* exudates. The fluctuation in the sex hormonal concentrations was attributed to the presence of certain bioactive compounds in the culture filtrate. These compounds might have a dysregulative effect on the reproductive hormones. In our previous study, it was shown that the injection of mice with the exudates of certain cyanobacterial species (*Nostoc ellipsosporum*, *Nostoc muscorum*, *Anabaena oryzae* and *Anabaena* sp.) led to a marked disruption in the serum level of the reproductive hormones[25]. At the same time, Ding *et al.* and Damkova *et al.* demonstrated that the exposure of vertebral models to the cyanobacterial extract induced negative consequences on the reproductive system such as sex hormones in addition to damaged testes and low quality of mature sperm[33,34].

The GC-MS and LC-MS/MS analysis showed the presence of sterol-like compounds in the exudates of *Spirulina*. In accordance with that, some studies clarified the existence of steroidal compounds such as cholesterol, beta-sitosterol, campesterol and stigmasterol in the dry mass of *Spirulina*[35,36]. Moreover, Ramadan *et al.* identified the lipid profile of *S. platensis* and clarified the existence of esterified sterols and certain free sterols in their extract[37].

The presence of sterol-like compounds in the culture filtrate of *Spirulina* might be responsible for the disruption of sex hormones via interfering with the hormone-dependent signaling pathways. Sterol-like compounds can increase or block the metabolism of naturally occurring steroid hormones via activating or antagonizing nuclear hormone receptors[38]. Moreover, these compounds may cause perturbations in the level of gonadotropin-releasing hormone which in turn affect the synthesis and the release of FSH and LH[39].

Regarding the level of neurotransmitters in the hippocampus region, the obtained results verified that both DA and NA levels were slightly elevated while a significant decline in 5-HT and Ach levels was observed in mice treated with *Spirulina* exudates. The fluctuation of neurotransmitters levels was correlated with the change in the level of sex hormones in the treated mice due to the effect of the sterol-like compounds of the culture filtrate. These results are in agreement with Cornil *et al.* who demonstrated that the injection of estradiol rapidly activated male sexual behavior in quail[40]. This effect was associated with changes in the concentration of monoamines and their activity. Additionally, Tucci *et al.* studied the effect of stanozolol, a synthetic anabolic steroid derived from dihydrotestosterone, on the level of monoamines in rat brain[41]. The treated rats showed neurochemical modifications of DA in hippocampus and prefrontal cortex while 5-HT was changed in all brain areas. In addition, the decline of Ach in response to sex hormonal alteration was in agreement with Takase *et al.* who supported the correlation between sex hormonal change and Ach level preservation in hippocampus[42].

The treated mice exhibited neurological symptoms comprising ataxia, convulsion and comma that were recovered one hour after each injection. These signs might be attributed to perturbation in monoamine neurotransmitters. It has been reported that change in monoamines was observed in various neurological and psychiatric disorders such as addiction, depression and attention

deficit/hyperactivity disorders[43]. Consequently, the disruption of sex hormones level could exert direct or indirect effect on the mammalian central nervous system either via regulating the expression of specific hormone-sensitive genes or by modulating the activity of neurotransmitter receptors[44,45].

The current study indicated a remarkable decrease in the level of the antioxidant enzymes (SOD, CAT and GPx) in the hippocampus of mice treated with *Spirulina* exudates. Under normal physiological conditions, there is a critical balance in the generation of oxygen free radicals and antioxidant defense system used by organisms to deactivate and protect themselves against free radical toxicity. As a result of the interference of the steroidal compounds identified in the *Spirulina* filtrate with sex hormone receptors, a marked disruption in the level of the sex hormones was observed. The alterations of the steroidal hormones concentration might be responsible for the reduction of the antioxidant enzymes activity. This hypothesis is consistent with Pajovic and Saicic who showed that the endogenous patterns of antioxidant defense enzyme expression are modulated by sexual steroid hormones[46].

Furthermore, Pajovic *et al.* and Michos *et al.* demonstrated that the enzyme activity of the antioxidant defense system in the brain tissue of female and male rats indicated a certain dependence on the concentration of steroidal hormones such as progesterone and estrogen[47,48]. Moreover, the decline in the abovementioned antioxidant enzymes along with Ach level as a response for the injection of *Spirulina* exudates might be an indication for the neuroinflammation and cholinergic neuronal degeneration in the hippocampus region. This hypothesis agrees with Sajad *et al.* and Santos *et al.* who demonstrated a remarkable inhibition of choline acetyl transferase, the enzyme responsible for Ach synthesis, in addition to significant decline of SOD and CAT in an animal model of human multiple sclerosis[49,50].

The current study demonstrated that the intra-peritoneal injection of *Spirulina* exudates in mice led to remarkable disruption of the reproductive hormones. This effect was attributed to the presence of steroidal compounds that were identified with GC-MS and LC-MS/MS analysis. The presence of such these compounds and their disruptive effects on sex hormones level were correlated with the fluctuation of monoamine neurotransmitters in the hippocampal region of the treated animals. The recorded neurological symptoms (ataxia, convulsion and comma) were attributed to the change in the level of monoamine neurotransmitters. At the same time, the reduction of the antioxidant enzymes activity and Ach level in the same region might be accompanied with hippocampal neurodegeneration due to the deteriorative effect of *Spirulina* exudates treatment. Consequently, *Spirulina* could potentially contribute to hazardous effects on mammalian health via producing sterol-like compounds that might disrupt endocrine system as well as the nervous system.

Conflict of interest statement

We declare that we have no conflicts of interest.

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