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Spirulina platensis attenuates furan reprotoxicity by regulating oxidative stress, inflammation, and apoptosis in testis of rats

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ABSTRACT

Furan is a common food contaminant and environmental pollutant. *Spirulina platensis* (SP) is a blue–green algae extensively used as therapeutic and health supplements. This study aimed to explore the probable beneficial role of SP against the influence of furan on reproductive system of male rats. Adult male rats were divided into control, vehicle control, SP (300 mg/kg bwt/ day, 7 days), furan (16 mg/kg bwt/ day,30 day), SP/furan, furan/ SP and furan + SP groups. Hematology, sperm count, sperm morphology, serum testosterone (TES), luteinizing hormone (LH), follicle-stimulating hormone (FSH) and estradiol (E2) levels, reduced glutathione (GSH), malondialdehyde (MDA), testicular enzymes, and pro inflammatory cytokines were estimated. In addition, histopathology of testis and seminal vesicles and apoptosis were evaluated. Anaemia, leukocytosis, and reduced gonadosomatic index were observed in the furan treated group. TES, LH, FSH, E2, and GSH were significantly decreased following furan treatment. MDA, testicular enzymes, and pro inflammatory cytokines were significantly incremented in testis of furan treated rats. Furan induced apoptic changes in testis. SP significantly counteracted furan reprotoxic impacts, particularly at co-exposure. Conclusively, these findings verified that SP could be candidate therapy against furan reprotoxic impacts.

1. Introduction

In recent years, heat-induced food contaminants have attracted the attention of both the scientific community and the public. Furan (C_4H_4O; CAS-Nr. 110–00-9), a volatile heterocyclic organic "high-production-volume" chemical, is produced in the cooking of various common foods, like baked or fried cereal products, coffee, canned and jarred foods, infant formula, and baby food (Morehouse et al., 2008). Furan is commonly adopted as a synthetic intermediate and in the production of stabilizers, pesticides, and pharmaceuticals. Also, furan is the main component of tobacco smoke. Hence, furan has been found in smog, surface water, acid hydrolysates, industrial effluents, and steam distillates of pentose-containing agricultural by-products like oat hulls and corn cobs (Gill et al., 2010).

Furan is a compound of low polarity and hence can easily pass via biological membranes. It exerts both metabolic and cellular effects through the interaction with arylhydrocarbon receptor (AhR) linked with xenobiotic- (XRE) and antioxidant responsive (ARE) DNA elements (Nguyen et al., 2009). The results of extensive studies have found clearly that the exposure to furan results in an extensive range of toxicities including hepatotoxicity (Ramm et al., 2016), carcinogenicity (Von Tungeln et al., 2017), and ovarian damage (Uçar and Pandir, 2017).

In a recent screening survey, Galimova et al. (2015) reported an increased level of furans in the ejaculate of infertile males compared with fertile donors suggests a relationship between furan exposure and reproduction function. El-Akabawy and El-Sherif (2016) reported an oxidative damage in testis of rat orally administered 4 mg/kg/day furan for 5 days per week for 90 days.

Spirulina platensis (Arthrospira platensis, SP*)*, a microscopic multicellular filamentous blue green algae (Cyanobacterium), is a natural source of proteins, vitamins, macronutrients and micronutrients like amino acids, gamma linolenic acid, carotenoids, especially β-carotene,α-linolenic acid, phycocyanin and phycocyanobilin, chlorophyll, and xanthophyll phytopigments. The antioxidant activity of SP and its ability to scavenge hydroxyl radicals and to hinder lipid peroxidation have attracted the attention of several researchers (Karadeniz et al., 2008).

The SP consumption as a diet supplement has health benefits in preventing or controlling obesity, inflammation (Coskun et al., 2011),

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and cardiovascular disease (Khan et al., 2005). In addition, SP has radioprotective (Mohamed et al., 2014), nephroprotective (Saber et al., 2015), reproprotective (Farag et al., 2016), and hepatotprotective (Toughan et al., 2018) effects.

Notwithstanding, about a third of current world production of SP is now traded for animal feed application. Moreover, several studies reported the marked favorable role of SP in fertility, animal growth, and nutritional product quality (Holman and Malau-Aduli, 2013).

Spirulina platensis possesses distinctive nutritional qualities together with an exceptional role in the treatment of plethora of diseased conditions (Xiong et al., 2018). These results of recent studies have stimulated our interest in investigating the defensive effects of SP against furan-induced reprotoxicity in vivo in rat. Therefore, the current study aimed to determine whether SP could attenuate furan-induced testicular damage, by investigating the probable antagonistic actions of SP on several aspects of testicular dysfunctions, biochemical and pathological abnormalities in male rat testes induced by furan.

2. Material and methods

2.1. Chemicals

Spirulina platensis is a bright, blue-green tablet with a specific fragrance produced by power nutritional, Jin Shun, Guangzhou, Trading Co., USA. Each tablet contains 250 mg pure SP. The Moisture, protein and chlorophyll content were ≤ 7.0%, ≥ 60%, and 12 mg/g, respectively. SP was dissolved in distilled water. Furan (99%, CAS Number 110–00-9, Sigma Aldrich, St. Louis, MO) was mixed (using stir bar) with corn oil, then stored in brown glass vials at 4 °C. Fresh solutions were prepared every week as needed. All other reagents and chemicals used were of analytical grade and purchased from Sigma- Aldrich Co. (St. Louis, MO, USA)

2.2. Animals

Healthy adult male Sprague-Dawley rats (n = 70 and average body weight of 180–200 g) were used in this study. They were obtained from the Laboratory Animal Housing Unit, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt. The animals were clinically healthy and kept under hygienic conditions in stainless steel cages with hard wood shavings as bedding. They were kept on basal ration, given water ad libitum and subjected to 12 h light-darkness cycle for 2 weeks of acclimatization before use.

The experimental procedures were performed in parallel to the general guidelines of the National Institutes of Health (NIH) for the Care and Use of Laboratory Animals in scientific investigations and approved by the ethics of animal use in research committee (EAURC), Zagazig University.

2.3. Experimental design

Rats were randomly distributed into seven experimental groups; each of 10 as follows: control group: received distilled water. Vehicle control group: received corn oil by gavage 5 days per week for four weeks. *Spirulina platensis* group (SP): received 300 mg/kg body weight SP, once daily for four weeks via gastric tube (Simsek et al., 2009). Furan group: orally administered with 16 mg/kg BW, once daily over four weeks (McDaniel et al., 2012). Protective group (SP/furan): orally administered with SP for one week, then with furan for four weeks. Therapeutic (furan/SP): orally administered with SP following (one week) the furan exposure as the same previously mentioned doses. Co exposure group (furan + SP) orally administered with SP and furan for four weeks.

2.4. Sampling

By the end of the dosing, two separate blood samples were collected from the retro-orbital plexus from each rat: the first blood sample was collected into an EDTA tube for hematological assessments. The second blood sample was collected into a tube (without EDTA) and left at room temperature for 20 min to coagulate; after centrifugation at 3000 rpm for 10 min, the resulted serum was isolated and placed at − 20 °C until used (within 2 weeks) for the estimation of sex hormones, including testosterone (TES), follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estradiol (E2). Seminal fluid was obtained from the epididymal tails for semen analysis. The testes and seminal vesicles were immediately removed. One testis of each rat was homogenized (1:4) in cold phosphate buffered saline (PBS, 0.01 mol/L, pH 7) with a glass homogenizer. The resultant homogenates were centrifuged at 5000 × *g* for 5 min; the supernatants were filtered through a Millipore filter (0.45 μm) to remove tissue debris and then used for measurement of oxidative stress assay, testicular enzymes and pro-inflammatory cytokines levels. The other was fixed in 10% buffered neutral formalin for histopathological and immunohistochemical staining.

2.5. Hematological evaluation

Using a Hema Screen 18 automated hematology analyzer (Hospitex Diagnostics, Sesto Fiorentino, Italy), total red blood cells (RBC), hemoglobin (Hb), packed cell volume (PCV), mean cell volume (MCV), mean corpuscular hemoglobin concentration (MCHC), total leukocytes, granulocytes, lymphocytes, and monocytes were determined

2.6. Semen evaluation

The cauda epididymis was carefully excised from the testis to release all the sperms. It was then directly placed in a vial with 5 mL prewarmed saline at 37 °C and minced via sterile scissors to get the seminal solution ready for analysis. A drop of freshly prepared seminal suspension was adopted to measure the percentage of motility via a high-power light microscope. The percentage of live sperms was estimated microscopically on eosine nigrosin-stained seminal films. Sperm abnormalities were recorded via the method of Filler (1993). Sperms were counted using a hemocytometer counting chamber after transferring 200 μL of the sperm suspension using an automatic pipette into a graduated test tube containing 800 μL of formolesaline to kill the spermatozoa. The sperm count was expressed as the total number of spermatozoa/mL.

2.7. Hormonal assay

Serum LH, TES, FSH, and E2 were determined via rat-specific enzyme-linked immunosorbent assay (ELISA) commercial kits (Cat. No.: MBS026898 for TES, MBS2509833 for LH, MBS703380 for FSH, and MBS263466 for E2) from My BioSource (San Diego, CA, USA) with the method of (Zirkin and Chen, 2000).

2.8. Testicular enzymes evaluation

Acid phosphatase (ACP) was assayed using enzyme-linked immunosorbent assay (Elisa) kits (MBS046840) following the manufacturer's instructions (MyBioSource, San Diego, CA, USA). Lactate dehydrogenase (LDH) diagnostic kit was supplied by Spinreact Co., Santa Coloma, Spain.

2.9. Oxidative stress assay

GSH estimations were made via the protocol described by Beutler (1963). Lipid peroxides (malondialdehyde, MDA) was estimated in testes homogenate by colorimetric assay as described previously