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Original article

Antagonistic effects of *Spirulina platensis* against sub-acute deltamethrin toxicity in mice: Biochemical and histopathological studies

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ABSTRACT

Spirulina platensis (SP); a microalga with high antioxidant and anti-inflammatory activities, acts as a food supplement in human and as many animal species. Deltamethrin (DLM) is a synthetic pyrethroid with broad spectrum activities against acaricides and insects and widely used for veterinary and agricultural purposes. Exposure to DLM leads to hepatotoxic, nephrotoxic and neurotoxic side effects for human and many species, including birds and fish. The present study was undertaken to examine the potential hepatoprotective, nephroprotective, neuroprotective and antioxidant effects of SP against sub-acute DLM toxicity in male mice. DLM intoxicated animals revealed a significant increase in serum hepatic and renal injury biomarkers as well as TNF-α level and AChE activity. Moreover, liver, kidney and brain lipid peroxidation and oxidative stress markers were altered due to DLM toxicity. Spirulina normalized the altered serum levels of AST, ALT, APL, LDH, γ-GT, cholesterol, uric acid, urea, creatinine AChE and TNF-α. Furthermore, it reduced DLM-induced tissue lipid peroxidation, nitric oxide and oxidative stress in a dose-dependent manner. Collectively, that Spirulina supplementation could overcome DLM-induced hepatotoxicty, nephrotoxicity and neurotoxicity by abolishing oxidative tissue injuries.

1. Introduction

Spirulina platensis (SP) is a microalga belonging to the class of cyanobacteria with a special formula of active constituents, including minerals, vitamins and protein, beta-carotene, tocopherols, phenolic acids, and excreting high anti-inflammatory and antioxidant activities, so it used as a food supplement in human, and feed additives in many animal species as well as birds and fishes [1,2]. Moreover, SP and its highly active ingredient; C-phycocyanin exhibit anti-inflammatory, immunomodulatory, hepatoprotective, nephroprotective, neuroprotective, antidiabetic, antigenotoxic, anti-hypertensive and anticancer activities [3–5]. Furthermore, SP has been reported to protect against many organ toxicities induced by heavy metals [6–10].

The extensive use of pesticides has been accompanied by environmental and human impact with high levels of hepatic and renal failure, infertility, cancer, immunosuppression and nervous diseases. The risks connected with the utilization of the earlier organophosphorus insecticides prompted the production of a new class of pesticides; synthetic pyrethroids to be important substitutes [11,12].

Deltamethrin (DLM) is a broad-spectrum synthetic type II pyrethroid insecticide [α-cyano-3-phenoxybenzyl-(1R,S)-cis, *trans*-3-(2,2-dibromovinyl)2,2dimethylcyclopropanecarboxylate], widely used to protect vegetables, fruits and agricultural crops, against pests such as mites, ants, beetles and weevils. It is also used for golf courses, nurseries, urban structural and landscaping sites, residential home and garden pest control. Moreover, it is utilized in

Abbreviations: BW, body weight; DLM, deltamethrin; SP, *Spirulina platensis*; MDA, malondialdehyde; NO, nitric oxide; CAT, catalase; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; GSH, reduced glutathione; TAC, total antioxidant capacity; ROS, reactive oxygen species.

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veterinary practice as an ectoparasiticide against flies, mites, ticks and fleas to control vector-borne illnesses [13]. It has become of choice in most countries because of its high power against a large number of pests, rapid breakdown and low toxicity to humans as well as non-target animals [14]. DLM represents an industrial and environmental pollutant that is toxic to fishes, birds, animals and human being living in the same biological community, and directly or indirectly at the danger of introduction prompting generous perils [15].

According to some reports, the liver was found to accumulate many metabolites since it is the principle site of DLM metabolism, and the kidneys are considered as the main excretory organ in mice and rats [16–19]. A number of studies on the side effects of this insecticide have been reported, including hepatotoxicity and nephrotoxicity [18–20], neurotoxicity [21], cardiovascular [22], immunosuppression [23–25] and reproductive side effects [26]. Although many reports about the sub-acute toxicity of DLM have been published [13,14,19], little studies have been done on the natural products for overcoming this toxicity, and mechanism of their ameliorative action. We hypothesize that natural products coadministration with DLM might ameliorate its toxic effects, the objective of the present study was to evaluate the antagonistic role of SP against subacute DLM-induced hepatotoxicity, nephrotoxicity and neurotoxicity in mice.

2. Materials and methods

2.1. Chemicals

Pure *S. platensis* powder was obtained from HerbaForce, UK. Deltamethrin is commercial available (Butox® 50 mg/ml), and was purchased from Intervet Co., France. Lactate dehydrogenase (LDH) kit was purchased from Randox Laboratories Ltd., U.K. All other kits were obtained from Biodiagnostics Co. (Cairo, Egypt). All other chemicals, which used in the current study, were of analytical grade.

2.2. Animals and experimental design

Thirty two male Swiss Albino mice, weighing 30 ± 3 g, were purchased from The Egyptian Organization for Biological Products and Vaccines. Mice were kept in ventilated room under controlled laboratory conditions of normal light–dark cycle (12 h light/dark) and temperature (25 ± 2 °C). Food and water were provided ad libitum. The animal handling and experimental design were approved by the Research Ethical Committee of the Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt (the approval no. 20146). All efforts were made to minimize animal suffering.

After 1week acclimation period, mice were randomly assigned to four different groups; eight animals each. The 1st group administered normal saline and serve as a control. The 2nd group was given DLM at a dose of 15 mg/kg (1/10 LD_{50}) [27]. The 3rd and 4th groups were treated with SP at doses of 500 and 1000 mg/kg respectively 1 h before DLM administration at the same dose of group 2. All treatments were given orally using stomach gavage, and continued for 30 days.

2.3. Serum collection and tissue preparation

At the end of experiment (24 h after last DLM administration), blood samples were collected via direct heart puncture, clot at room temperature, and then centrifuged at 3000 rpm for 15 min. Sera were next, separated and kept at −20 °C as aliquots for further biochemical analysis.

After blood collection, mice were sacrificed by decapitation. Liver, kidney and brain were rapidly excised from each animal, trimmed of the surrounding tissue, and washed with 0.9% NaCl solution and distilled water. They were then blotted over a piece of filter paper. The tissues were perfused with a 50 mM (sodium phosphate buffer saline (100 mM Na2HPO4/NaH2PO4) (PH 7.4) in an ice-containing medium, containing 0.1 mM ethylene di amine tetra acetic acid (EDTA) to remove any red blood cells and clots. Then tissues were homogenized in 5–10 ml cold buffer per gram tissue and Centrifuged at 5000 r.p.m for 30 min. The resulting supernatant was transferred into Eppendorf tubes, and preserved at −80 °C in a deep freezer until used for various biochemical assays.

2.4. Serum biochemical analysis

The sera were used for estimation of serum liver and renal injury markers according to manufacturer protocol. Serum aminotransferases, AST and ALT were evaluated according to [28], Alkaline phosphatase (ALP) according to [29]. Lactate dehydrogenase (LDH) was determined or the quantitatively in serum according to the method of [30]. Gama glutamyl transferase (γ-GT) was evaluated according to [31]. Cholesterol was measured according to [32,33]. Renal products; creatinine was evaluated according to [34], urea according to [35] and uric acid according to [36]. Acetylcholinesterase (AChE) was determined by according to the method of Ellman et al. [37].

2.5. Evaluation of tissue lipid peroxidation and antioxidant enzymes

Malondialdehyde was evaluated in hepatic, renal and brain tissues as a measure of tissue lipid peroxidation according to [38]. Nitric oxide (NO) content in the tissues was determined according to [39]. Antioxidant markers were assessed; superoxide dismutase (SOD) according to [40], catalase (CAT) according to [41], reduced glutathione (GSH) according to [42], glutathione peroxidase (GSH-Px) according to [43].

2.6. Cytokine assays

Serum TNF-α was measured using already made kits from Assay Designs Inc. (Ann Arbor, MI, USA) using The Assay Max Mouse TNF-α ELISA kit according to manufacturer instructions through a quantitative sandwich enzyme immunoassay protocol. Briefly, a specific mouse monoclonal antibody specific has been pre-coated onto 96-well microtiter plates. The cytokine in the specimens and standards is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for the cytokine, which is recognized by a streptavidin–peroxidase conjugate. All free non-specific material was then washed away, and a peroxidase enzyme substrate is added. Subsequently, the produced color was halted, and the plates were read at 490 nm using an ELISA reader.

2.7. Histopathological examination

Liver, kidney and brain sections were taken immediately, fixed in 10% buffered formalin, dehydrated gradually using ethanol (50–100%), and embedded in paraffin after being cleared in xylene. Sections (4–5 μm thick) were prepared, and then stained with hematoxilin and eosin (H–E). The sections were examined for the pathological findings.

2.8. Statistical analysis

All data were expressed as means ± SE, and were analyzed using one-way ANOVA with Duncan's post hoc test using SPSS version 20.0 (SPSS Inc., Chicago, IL), to determine the significant