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Original article

Antioxidant effects of Spirulina supplement against lead acetate-induced hepatic injury in rats

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ABSTRACT

Lead is a toxic metal that induces a wide range of behavioral, biochemical and physiological effects in humans. Oxidative damage has been proposed as a possible mechanism involved in lead toxicity. The current study was carried out to evaluate the antioxidant activities of Spirulina supplement against lead acetate -induced hepatic injury in rats. Five groups of rats were used in this study, Control, Lead acetate (100 mg/kg), Lead acetate (100 mg/kg) + 0.5 g/kg Spirulina, Lead acetate (100 mg/kg) + 1 g/kg Spirulina and Lead acetate + 25 mg/100 g Vitamin C (reference drug). All experimental groups received the oral treatment by stomach tube once daily for 4 weeks. Lead intoxication resulted in a significant increase in serum alanine transaminae (ALT), aspartate transaminae (AST) activities, liver homogenate tumor necrosis factor-α (TNF-α), caspase-3, malondialdehyde (MDA), nitric oxide (NO) levels and a significant decline of total serum protein, liver homogenate reduced glutathione (GSH) level and superoxide dismutase (SOD) activity. Both doses of Spirulina supplement as well as Vitamin C succeeded to improve the biochemical parameters of serum and liver and prevented the lead acetate-induced significant changes on plasma and antioxidant status of the liver. Both doses of Spirulina supplement had the same anti-apoptotic activity and high dose exhibited more antioxidant activity than that of low dose. In conclusion, the results of the present work revealed that Spirulina supplement had protective, antioxidant and anti-apoptotic effects on lead acetate-induced hepatic damage.

1. Introduction

Lead (Pb) is a toxic metal that induces a wide range of behavioral, biochemical and physiological effects in humans. Even though blood lead levels continue to decline over the past two decades, specific populations like infants, young children and working class are still at a higher risk.[1] Childhood lead exposure is estimated to contribute to about 600 000 new cases of children developing intellectual disabilities every year. Lead exposure is estimated to account for 143 000 deaths per year with the highest burden in developing regions. About one half of the burden of disease from lead occurs in the WHO South-East Asia Region, with about one-fifth each in the WHO Western Pacific and Eastern Mediterranean Regions.[2] As lead exposure tends to be sub acute, produces only subtle clinical symptoms. Chronic exposure cases are more common than acute toxicity. Lead via gastro intestinal absorption is first taken up by the red blood cells and is distributed to all vascular organs.[3] Pathogenesis of lead poisoning is mainly attributed to lead-induced oxidative stress. Chronic lead exposure is known to disrupt the pro oxidant/antioxidant balance existing within the mammalian cells.[4,5] Lead is reported to cause oxidative stress by generating the release of reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide and hydroxyl radicals and lipid peroxides. There has been increased interest among phytotherapy researchers to use medicinal plants with antioxidant activity for protection against heavy metal toxicity.[6–8]

Pharmaceutical drugs that depend on compounds such as colchicine and interferons are currently available for the treatment of liver cirrhosis but with either unreliable efficacies or high situations of side effects.[9]

A number of natural compounds produced from vegetation offer alternative healthcare options that are more effective and safe.[10] Ingredients from recently found or already known plant varieties

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are regularly being examined on experimental animals.[11] The prospective tasks and efficiency of plant extracts in liver diseases are yet to be analyzed. Spirulina Slimming Capsule, the product chooses the natural herbal product combining *Spirulina platensis* and *Aloe* extract.

Spirulina is a great source of natural protein (about 60% digestible proteins). It is a low fat, low calorie, cholesterol free source of protein with all amino acids, phyto-nutrients, antioxidants, carbohydrate, muco-polysacchrides, vitamins and trace minerals, an exclusive ingredient, prevents the digestion of dietary fat, hence reducing its absorption by the body. After sometime of consumption, body parts prone to fat accumulation (belly, arms, thighs and buttoks) could see dramatic benefits. Spirulina soft capsule promote gastrointestinal motility, remove intestinal toxins, improve constipation. Spirulina Slimming. It can reduce the absorption of lipophilic analogs by intestinal tract, control the ingestion of heat quantity, avoid the storage of fat, plug up the source of fat and burn the quondam fat by the way of heat production. It accelerate the metabolic rate of fat, which change the fat into sugar and protein, to protect the nutrition that people need and keep slimming. In addition, it has the function of hairdressing. Many people find it effective as a natural appetite suppressant. It is also known to assist blood circulation. The present study was planned to investigate the antioxidant, heptoprotective and apoptotic effects of Spirulina supplement against lead-induced hepatic injury and apoptotic changes in rats.

2. Materials & methods

Spirulina Slimming, Fat Burner Capsules 350 mg (China go2slimming LLC NO.315 Kunming Luosiwan Commercial Center Kunming, Yunnan, China 650214) was purchased from private Pharmacy, Cairo, Egypt.

2.1. Experimental design

Thirty male Wistar albino rats weighing (140–160) g were used for this study. The animals were housed in a temperature (25 ± 1 °C), humidity controlled room and a 12-h light-dark cycle. Rats were allowed free access to tap water and standard pellet diet. The institutional Animal Ethics Committee approved all experimental protocols. The animals were classified into 5 groups, each of 6 as follows:

Control group (C): Rats received distilled water.
Lead acetate-treated group (LA): Rats were orally administered lead acetate at a dose of 100 mg lead acetate/kg body weight, by stomach tube once daily for 4 weeks.
Lead acetate and 0.5 g/kg Spirulina treated group (LA + 0.5 g/kg Spirulina): Rats were orally administered lead acetate at a dose of 100 mg lead acetate/kg body weight and 0.5 g Spirulina/kg body weight by stomach tube once daily for 4 weeks.
Lead acetate and 1 g/kg Spirulina treated group (LA + 1 g/kg Spirulina): Rats were orally administered lead acetate at a dose of 100 mg lead acetate/kg body weight and 1 g Spirulina/kg body weight by stomach tube once daily for 4 weeks.
Lead acetate and 25 mg/100 g Vitamin C(reference drug) treated group (LA + 25 mg/100 g Vitamin C): Rats were orally administered 100 mg lead acetate/kg body weight and 25 mg Vitamin C/100 gm body weight by stomach tube once daily for 4 weeks.

At the end of experiment, fasting blood samples were withdrawn from the retro-orbital vein of each animal using a glass capillary tube after fasting period of 12 h. The blood samples allowed to coagulate and then centrifuged at 3000 rpm for 20 min. The separated sera were used for the estimation of serum activities of ALT and AST by using commercial kits (Quimica Clinica Aplicada, Spain). Total serum protein was evaluated using kits from Biodiagnostic, Egypt.

2.2. Preparation of liver homogenate

A portion of liver was excised, accurately weighed and homogenized in ice-saline to prepare a 10% (w/v) tissue homogenate. The homogenate was used for the determination of GSH level, SOD activity, end product of lipid perioxidation, MDA and NO levels.

The protein content of liver homogenates was evaluated by the method of Lowry *et al*[12] and using bovine serum albumin as a standard. GSH level in liver tissue was measured by the method of Ellman using 5, 5′-dithiobis-(2-nitrobenzoate) at 412 nm.[13]

SOD activity in liver tissue was determined by the method of Marklund and Marklund[14] pyrogallol (24 mmol/L) was prepared in 10 mM HC1 and kept at 4 °C Before use. Stock catalase solution (30 μmol/L) was prepared in phosphate buffer (pH 9, 0.1 M), 100 μl of the supernatant was added to Tris HC1 buffer (pH 7.8, 0.1 M) containing 25 μl pyrogallol and 10 μl catalase. The final volume was adjusted to 3 ml using the same buffer solution. Changes in the absorbance at 420 nm were recorded at 1 min interval for 3 min. Data were expressed as U/mg protein.

In liver homogenate, MDA, a stable product of lipid peroxidation was estimated by method of Ohkawa.[15] In brief, 0.5 ml of homogenate was mixed with 2.5 ml of 20% trichloroacetic acid and centrifuged at 3000 rpm for 10 min. The supernatant was decanted and the precipitate was washed once with 0.05 M sulphuric acid and then 3 ml of 0.2 g/dl thiobarbituric acid reagent was added to the precipitate. The mixture was heated in a boiling water bath for 30 min. After cooling in cold water, the resulting chromogen was extracted with 4 ml of *n*-butyl alcohol. The organic phase was separated by centrifugation at 3000 rpm for 10 min and absorbance was recorded at wavelength of 530 nm.

NO level in liver tissues was measured by using the commercial kits supplied by Biodiagnostic, Egypt. The assay is based on the diazotization of sulfanilic acid with nitric oxide at acidic pH and subsequent coupling with N-(1-naphthyl)-ethylenediamine to yield an intensely pink colored product that was measured spectrophotometrically at 540 nm. Sodium nitrite was used as standard.

2.3. Estimation of TNF-a and caspase-3 levels

A portion of the liver was weighed (50 mg) and homogenized in 0.8 ml lysis buffer, pH 7.4. The lysate was centrifuged at 10 000 g for 15 min at 4 °C, and the supernatant was taken for estimation of the TNF-α and caspase-3 levels by a sandwich enzyme immunoassay kit (Cloud Clone Corp.) for in vitro quantitative measurement of TNF-α and caspase-3 level in rat tissue homogenates.

2.4. Statistical analysis

Results were shown as mean ± S.E. for each group. Statistical analysis was performed using SPSS 9.0 for Windows (Chicago, IL, USA). For multiple comparisons, one-way analysis of variance (ANOVA) was used. In cases where ANOVA showed significant differences, Tukey test was performed. $P < 0.05$ was considered to be statistically significant.

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