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Original article

Hepatoprotective and antioxidative effects of C-phycocyanin from *Arthrospira maxima* SAG 25780 in CCl_4-induced hepatic damage rats

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ABSTRACT

The efficacy of C-phycocyanin obtained from *Arthrospira maxima* SAG 25780 was evaluated on the levels of lipid peroxidation, and antioxidant parameters as well as histopathological and immunohistochemical assessment in carbon tetrachloride (CCl_4) induced liver damage in the male Wistar albino rats. The rats were injected by CCl_4 intraperitoneal (50% CCl_4, 0.5 mg/kg body weight) that led to a marked increase in oxidative stress indicated by alterations in lipid peroxidation, enzymic and non-enzymic antioxidants. Treatment with C-phycocyanin (75 mg/kg body weight) restored the above-mentioned alterations towards normalcy, highlighting the antioxidant potential of C-phycocyanin in mitigating the oxidative stress mediated damage produced during CCl_4-induced male rats. The liver damage was further confirmed by histopathological and immunohistochemical studies. The present investigation strongly suggested that the C-phycocyanin protects CCl_4-induced hepatotoxicity in Wistar rats.

1. Introduction

The Cyanobacterium *Arthrospira platensis* has been used by humans because of its nutritional and potential medicinal effects. *Spirulina* deserves special attention both as a source of single cell protein (SCP) [1] and for its nutraceutical properties such as provitamins, minerals, proteins and polyunsaturated fatty acids such as α-linolenic acid [2].

Spirulina extracts can prevent or inhibit cancer in humans and animals. *In vitro* studies suggest that polysaccharides of *Spirulina* enhance cell nucleus enzyme activity and DNA repair synthesis. *Spirulina* is a powerful stimulant for the immune system [3] as shown in animal experiments, by increasing the phagocytic and the natural killer activities. C-phycocyanin a biliprotein from *Spirulina platensis* is a selective inhibitor of cyclooxygenase-2 (COX-2). Apoprotein in phycocyanin plays a key role in the selective inhibition of COX-2. The hepatoprotective, anti-inflammatory and anti-arthritic properties of phycocyanin reported may be due to its selective COX-2 inhibitory property, although its ability to efficiently scavenge free radicals and effectively inhibit lipid peroxidation may also are involved [4].

S platensis could be used as a dietary supplement to prevent some diseases where free radicals are involved. The *in vitro* scavenger activities of different reactive oxygen species (superoxide radical, hydroxyl radical, hydrogen peroxide (H_2O_2), hypochlorous acid and peroxyl radical) and the effect on enzymatic or non-enzymatic lipid peroxidation were studied [5]. Oxidative stress conditions can cause DNA and protein damage, lipid peroxidation, cancer, atherosclerosis, ageing and inflammatory diseases.

Carbon tetrachloride (CCl_4) is largely used as solvent in chemical industries. CCl_4 is well known for hepatic and renal toxic actions. The metabolism of CCl_4 into trichloromethyl ($CCl_3\bullet$) and peroxy trichloromethyl ($\bullet OOCCl_3$) free radicals has been reported to cause acute liver damage like cirrhosis, steatosis and necrosis [6]. To our knowledge, no report is available until now, for the protective action of purified cyanobacterial phycocyanin (C-PC) on CCl_4-induced toxicity *in vivo*. The present investigation deals with dose-responsive treatment of C-PC against CCl_4-induced hepatic toxicity in rats by comprehensive evaluation of hepatocellular and oxidative biomarkers.

2. Materials and methods

Arthrospira maxima SAG 25780 was obtained from the culture collection of algae, Centre for Advanced Studies in Botany, University of Madras, Chennai, India it was maintained in half-strength

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Table 1
Effect of C-phycocyanin on the concentration of antioxidant enzymatic assay.

Parameters	Group I	Group II	Group III	Group IV
SOD	8.72 ± 0.35	5.30 ± 0.31[a]	9.58 ± 0.47[b]	7.85 ± 0.35
CAT	63.4 ± 5.13	47.1 ± 4.61[a]	62.8 ± 5.65[b]	58.72 ± 4.99
GPx	98.4 ± 8.06	65.6 ± 6.36[a]	101.7 ± 8.94[b]	89.32 ± 7.23
GR	162.33 ± 12.98	122.9 ± 11.92[a]	164.01 ± 14.27[b]	143.10 ± 12.02

(Group I-control); (Group II-CCl_4); (Group III-CCl_4 + phycocyanin); (Group IV-phycocyanin alone). Effect of C-phycocyanin on the concentration of low molecular weight antioxidants are mean ± S.D. for six animals for each group. The symbols '*a*' and '*b*' represent significance at $P < 0.05$, where a = compared with Group I and b = compared with Group II. GPx: glutathione peroxidase; GR: glutathione reductase; SOD: superoxide dismutase; CAT: catalase.

[7] and kept under 30 $\mu Em^{-2}s^{-1}$ light irradiance, 12/12 h light/dark cycle and 24 ± 1 °C. Optimally grown 16 days old culture was taken for investigations.

2.1. Extraction of C-phycocyanin

The selected cyanobacterial sample was harvested after 15 days of inoculation under laboratory conditions and its extraction of C-phycocyanin following the method [8] and it's purified samples was used for drugs against CCl_4-induced rats.

2.2. Experimental design

Male Wistar rats weighing appropriately 180–200 g were obtained from Tamil Nadu Veterinary and Animal Sciences University, Chennai, India. Animals were housed in polypropylene cages in controlled room. The animals were supplied with standard pellet diet (Hindustan lever limited, Bangalore, India) and water *ad libitum***.** The animals were used for the study on approval by the Institutional Animal Ethical Committee (IAEC No. 03/017/09).

The animals were divided into four groups of six animals each.

Group I: control (normal diet); Group II: CCl_4-induced (50%, 0.5 mg/kg body weight/7 days i.p.); Group III: CCl_4-induced + phycocyanin treated; Group IV: phycocyanin treated (75 mg/kg b.wt.). After 24 h of administration of CCl_4, the animals were killed by cervical dislocation after an overnight fast. The liver tissue was used for the determination of the following parameters.

2.3. Biochemical studies

The liver were excised and perfused with ice-cold saline (0.9% sodium chloride). A 10% liver homogenate was prepared with fresh tissue in 0.1 M Tris-HCl buffer (pH 7.4).

Procedure

Four hundred milligram of the liver tissue was taken in 4 ml of homogenizing buffer. It was grinded in mortar and pestle in cold condition. Then the homogenate was centrifuged at 10,000 rpm for 15 minutes at −4 °C. The precipitate was discarded and supernatant was collected and stored in ice box. The tissue homogenate was used for estimation of protein content and different antioxidant parameters were analyzed for enzymatic and non enzymatic levels.

2.4. Assay of Thiobarbituric acid reactive substances (TBARS)

Lipid peroxidation was determined in the liver tissue and plasma by measuring the formation of thiobarbituric acid reactive substances (TBARS) according to the method of [9]. Hydroperoxides were estimated both in plasma and in tissue homogenate by the method described [10].

2.5. Assay of enzymic antioxidants

Superoxide dismutase (SOD) was assayed following the method [11]; catalase (CAT) was assayed according to the method [12]; glutathione peroxidase (GPx) activity was determined by the method [13] and glutathione reductase (GR) activity was measured by the method [14].

2.6. Estimation of non-enzymic antioxidants

Determination of total reduced glutathione (GSH) in the liver tissue was estimated by the method [15]. Estimation of α-Tocopherol (Vitamin E) was estimated by the method [16] the ascorbic acid (Vitamin C) content was determined by the method [17] and Vitamin A was estimated by the method [18] the values were expressed as μg/g tissue.

2.7. Histopathological studies

A portion of the liver was fixed in 10% formalin, processed by routine histology procedures, embedded in paraffin, cut in 5 μm pieces and mounted on the slide. The samples were stained with haematoxylin and eosin for histopathological examination. Each visual field was magnified at 40×. The average value of at least four different sections from four different rats was counted.

2.8. Statistical analysis

Values are expressed as mean ± SD for six rats in each group, and significant differences between mean values were determined by one-way analysis of variance (ANOVA) by Agres statistical software package (Agres, 1994). The Least Significant Difference (LSD) analysis was performed to group the treatment mean values.

3. Results

3.1. Enzymatic assays

The activities of enzymic antioxidants in liver tissue and hemolysate of control and experimental group of animals is shown in Table 1. A significant descends in the activities of SOD, CAT, GPx and GR was evident in CCl_4-induced rats (Group II) when compared with the control (Group I) and C-phycocyanin-treated rats (Group III). C-phycocyanin administration to CCl_4-induced rats significantly restored the activities of these enzymes as compared with CCl_4-induced group (Group II) with C-phycocyanin alone treated group (Group IV) exhibited more prominent activities of these enzymic antioxidants as compared with Group III).

3.2. Non-enzymatic assays

The levels of GSH in plasma and tissue of control and experimental groups of rats are shown in Fig. 1 and Table 2. A significant decrease in the levels of vitamin A, vitamin C and vitamin E was evident in CCl_4-induced rats (Group II) when compared with control (Group I) and C-phycocyanin treated rats (Group III). C-phycocyanin administration to CCl_4-induced rats significantly increased the levels of these antioxidants as compared with CCl_4-induced rats (Group II), with the C-phycocyanin treated rats (Group III) exhibited more pronounced effect when compared