

Hepatoprotective and Antioxidative Effects of C-Phycocyanin in CCL₄ Induced Hepatic Damage Rats

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Abstract: The efficacy of C-phycoerythrin 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 (CCl₄) induced liver damage in the male Wistar albino rats. The rats were injected by CCl₄ intraperitoneal (50% CCl₄, 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-phycoerythrin (75 mg/kg body weight) restored the above mentioned alterations towards normalcy, highlighting the antioxidant potential of C-phycoerythrin in mitigating the oxidative stress mediated damage produced during CCl₄ induced male rats. The liver damage was further confirmed by histopathological and immunohistochemical studies. In the present investigation, strongly suggested that the C-phycoerythrin protects CCl₄ induced hepatotoxicity in Wistar rats.

Key words: C-phycoerythrin • Histopathology • Immunohistochemistry • Carbontetrachloride

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-phycoerythrin 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-arthritis 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 be involved [4].

Spirulina 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, hypochlorous acid and peroxy 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. respond

Carbon tetrachloride (CCl₄) is largely used as solvent in chemical industries. Carbon tetrachloride is well known for hepatic and renal toxic actions. The metabolism of CCl₄ into trichloromethyl (CCl₃•) and peroxy trichloromethyl (•OOCCl₃) free radicals has been reported to cause acute liver damage like cirrhosis, steatosis and necrosis [6]. The present investigation deals with dose-responsive treatment of C-PC against CCl₄-induced hepatic toxicity in rats by comprehensive evaluation of hepatocellular and oxidative biomarkers.

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

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

Experimental Design: Male Wistar rats weighing appropriately 180-200g 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 (75mg/kg b.wt.). After 24 hrs 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.

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 0.1M Tris-HCl buffer (pH 7.4).

Procedure: Four hundred mg 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 15minutes 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.

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].

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].

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 $\mu\text{g/g}$ tissue.

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 40x. The average value of at least four different sections from four different rats was counted.

Statistical Analysis: Values are expressed as mean \pm 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.

RESULTS

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-phycoyanin-treated rats (Group III). C-phycoyanin administration to CCl_4 -induced

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-CCl₄); (Group III-CCl₄+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.

Table 2: Effect of C- Phycocyanin on the concentration of non-enzymatic antioxidant assay

Parameters	Group I	Group II	Group III	Group IV
Vitamin A	1.67±0.06	0.82±0.04 ^a	1.43±0.07 ^b	1.59±0.06
Vitamin C	2.31±0.09	0.94±0.05 ^a	2.19±0.12 ^b	2.29±0.09
Vitamin E	3.56±0.14	1.87±0.10 ^a	3.62±0.15 ^b	3.92±0.18

*(Group I - Control); (Group II - CCl₄); (Group III - CCl₄+Phycocyanin) and (Group IV - Phycocyanin alone). Effect of C- Phycocyanin on the concentration of low molecular weight antioxidants are mean ± SD 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.

Table 3: Levels of TBARS in liver and plasma of control and experimental groups of rats

Experimental Group	Liver TBARS mmol/100g tissue	Plasma TBARS nmol/mL
Control	37.85±3.10	6.36±0.32
Ccl ₄	36.93±2.95 ^a	28±0.21 ^a
Ccl ₄ +C-phycocyanin	67.21±6.58 ^b	9.65±0.53 ^b
C-phycocyanin	48.73±4.62	4.32±0.22

CCl₄-carbon tetrachloride; TBARS-thiobarbituric acid reactive substances.

Values are given as mean ± SD for groups of six rats each. Values are given statistically significant at $P < 0.05$. The symbols 'a' and 'b' represent significance at $P < 0.05$, where *a* = compared with Group I and *b* = compared with Group II.

rats significantly restored the activities of these enzymes as compared with CCl₄-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).

Non-Enzymatic Assays: levels of GSH in plasma and tissue of control and experimental groups of rats are shown in Fig. 2 and Table 2. A significant decrease in the levels of vitamin A, vitamin C and vitamin E was evident in CCl₄ induced rats (Group II) when compared with control (Group I) and C-phycocyanin treated rats (Group III). C-phycocyanin administration to CCl₄-induced rats significantly increased the levels of these antioxidants as compared with CCl₄-induced rats (Group II), with the C-phycocyanin treated rats (Group III) exhibited more pronounced effect when compared group (Group II). The recoument of vitamin A, C and E, to near normal level in Group IV was due to potent antioxidant activities of the C-phycocyanin.

Assay of Thiobarbituric Acid Reactive Substances (TBARS):

Table 3 shows the levels of TBARS in the liver tissue and plasma of control and experimental group of rats. Significantly elevated levels of TBARS were observed in CCl₄ - induced rats (Group II) as compared with the control (Group I) and C-phycocyanin treated animals (Group III). Treatment of C-phycocyanin to CCl₄-induced rats significantly lowered the liver tissue and plasma levels of TBARS as compared with CCl₄-induced rats; with more pronounced effect observed in C-phycocyanin alone group (Group IV).

Histopathological Studies:

Carbontetachloride (CCl₄) induces various centrilobular alterations in the hepatic tissue of rats. Liver tissues of control group showed normal architecture. Whereas, in CCl₄ induced and drug (C-phycocyanin) treated (Group-III) animals showed a lesser degree of damage in comparison with Group II animals. The Group III animal liver showed prominent

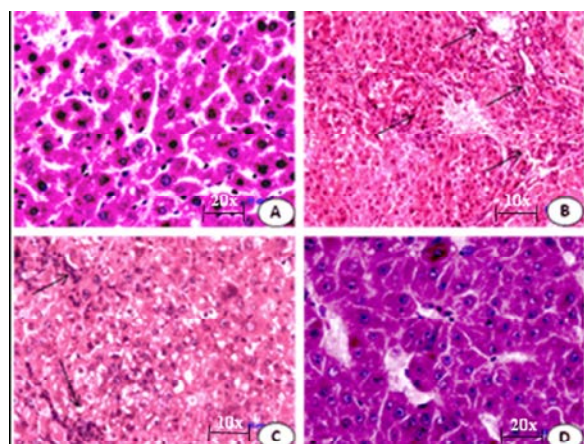


Fig. 1: Histopathological observation in liver of control and experimental groups of rats
 (A)Control (20x)
 (B)CCl₄-carbon tetrachloride (10x)
 (C)CCl₄+ C-phycoerythrin (10x)
 (D)C-phycoerythrin alone (20x)

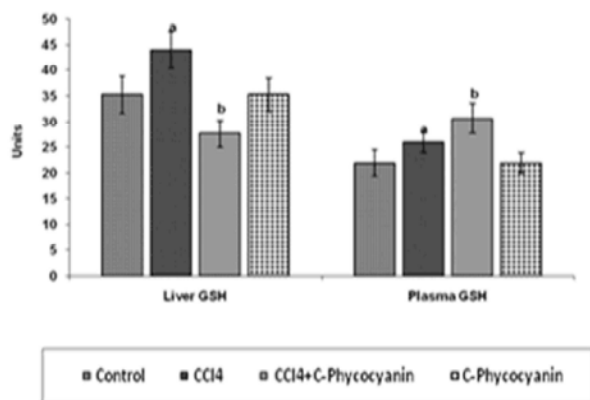


Fig. 2: levels of GSH in liver and plasma of control and experimental groups of rats
 GSH-reduced glutathione: Ccl₄-carbon tetrachloride:
 Units of liver GSH mg/100g tissue: Units of plasma GSH mg / dL.
 Values are given as mean ± SD for groups of six rats each. Values are given statistically significant at P<0.005. The symbols "a" and "b" represent significance at p< 0.05, where a = compared with Group I and b = compared with Group II.

recovery in form of normal hepatocytes and lesser centrilobular necrosis. Group IV drug alone (C-phycoerythrin) treated animals also showed normal architecture of liver tissue (Figure 1).

DISCUSSION

Liver is the key organ of metabolism and excretion is constantly endowed with the task of detoxification of xenobiotics, environmental pollutants and chemotherapeutic agents. This disorders associated with this organ are numerous and varied. CCl₄, a protoxical substance undergoes metabolism in the liver, resulting in the damage of liver cells, which is capable of producing toxic effects at sites far from liver. The C-phycoerythrin is an excellent antioxidant property and scavenging free radicals like superoxide and hydroxyl radicals.

The antioxidation of ROS and free radicals and decreased GSH levels signify increased oxidative stress [19]. It is widely accepted that the induction of antioxidant enzymes are a major strategy for protecting cells against a variety of endogenous and exogenous toxic compounds such as ROS and chemical carcinogens [20]. The overall effect of depletion of glutathione with concomitant increases in the lipid peroxide levels provides evidences about vital role played by glutathione during oxidative stress. The significant protection offered by C-phycoerythrin demonstrated the radical scavenging activity and its inhibitory effect on lipid peroxidation chain reaction. The observed increase in circulating lipid peroxidation of CCl₄ treated animals, in the present study, correlates with the decline circulatory antioxidants such as SOD, CAT, GPx, GR and GSH. These may be due to their over utilization to scavenge the products of lipid peroxidation as well as sequestration by injury cells. In addition GSH, Vitamin A, C and E scavenge free radicals to prevent lipid peroxidation of polyunsaturated fatty acids, which can act as promoters of liver damage and hepatoprotective action by up-regulating GSH-dependent enzymes and inhibiting free radical formation in the liver [21]. Lipid peroxides levels were significantly higher in CCl₄ induced rats when compared to control rats. The drug treated rats showed decreased LPO levels when compared to CCl₄ administrated rats, where only C-phycoerythrin was administrated, no significant changes were observed when compared to control. SOD and CAT are widely distributed in all cells and are present in high amounts in erythrocytes [22]. GPx catalyses the reduction of hydrogen peroxide (H₂O₂) and organic hydroperoxides at the expense of glutathione. GSH play a key role in several detoxification reactions and in reduction of lipid peroxides. GR plays a major role in regenerating GSH from GSSG, thus maintaining the balance between the redox couple [23].

Vitamin A as an antioxidant is involved in counteracting free radicals and is known to help in the repair of damaged tissues [24]. Vitamin A at pharmacological doses is reported to ameliorate oxidative stress mediated membrane lipid peroxidation and membranes enriched with vitamin A are protected against oxidative stress *in vivo* and exhibit resistance to LPO induced *in vitro* [25]. The vitamin C, an important antioxidant, acts in tissues, involving ROS in aqueous phase and it has been reported that the tissue concentration of vitamin C is a good indicator of oxidative stress [26]. Vitamin E is a principal lipid soluble antioxidant in cell membranes that protects critical cellular structures against oxidative damage [27]. The concentration of vitamin E has been inversely correlated to LPO [28].

The increased level of vitamin A, C and E in Group III as compared to Group II also indicate the antioxidant activity of C-phycoerythrin. The decreased levels of Vitamins A, C & E in CCl₄ administered animals may be due to increased utilization of these antioxidants to counter lipid peroxidation. Increased plasma concentrations of these vitamins in C-phycoerythrin animals may be because, C-phycoerythrin scavenged the free radicals involved in the process of cell damage thus enhancing the concentrations of plasma vitamins. The histopathological analyses of liver tissues were examined in the control and experimental groups of rats [29, 30]. Our results strongly suggested that administration of C-phycoerythrin during the initiation stages of liver injury significantly inhibited cell damage incidence, decreased circulatory lipid peroxidation and enhanced enzymic and non-enzymic antioxidant concentrations. The present study was revealed that C-phycoerythrin has potent effects and has emphasized its protective role against CCl₄ induced experimental Wistar rats.

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