

Effect of *Spirulina platensis* Food Supplement on Blood Morphological Parameters on Swiss Albino Male Mice

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ABSTRACT

In the present study, we have evaluated the hematological parameter effects of *Spirulina platensis* against CCl₄-induced hepatotoxicity in mice. CCl₄ induction (1 mg/kg b.wt), the results showed that significantly effect on hematological parameter (RBCs, Hb, WBCs and PLT). Treatment of *Spirulina platensis* (800 mg/kg/b.wt) to CCl₄ challenged mice.

Key words: Blue green algae; hematological parameter; mice.

Introduction

Spirulina or *Arthrospira* has attracted the attention of researchers for many years, as shown by the hundreds of publications in its various aspects. *Spirulina* (Blue green algae) is a microscopic single cell alga which grows in fresh water and has a simple structure but a complex composition. It is a concentrated source of food containing nutraceutical, antioxidants, probiotics properties. Moreover, it is an important source of the blue photosynthetic pigmented protein C-phycoyanin, which has strong antioxidant and anti-inflammatory properties. Interestingly, *spirulina* is known for its wide ranging biological activities, like prevention of anemia because of high iron and vitamin contents (Hemalatha *et al.*, 2012), inhibition of herpes simplexinfection (Ferreira-Hermosillo *et al.*, 2011), reduction in HIV replication velocity (Ayehunie *et al.*, 1998), increased production of antibodies, prevention of proliferation of neoplastic cells (Premkumar *et al.*, 2004), hypoglycemic (Parikh *et al.*, 2001; Abdel-Daim *et al.*, 2013), hypolipidemic (Jarouliya *et al.*, 2012) and antihypertensive properties in experimental animal and humans models (Ponce-Canchihuamán *et al.*, 2010), furthermore, it shows hepatoprotective properties through decreasing of the liver lipid profiles and lipoperoxidation products (Abd El-Baky *et al.*, 2009), antimutagenic, antiviral, immune enhancing, cardioprotective and anticancer properties (Khan *et al.*, 2005). The toxic metabolite CCl₄ radical is produced which is further converted to trichloromethylperoxy radical by cytochrome P450 2E1 enzyme. This radical binds covalently to the macromolecules and causes peroxidative degradation of cellular membrane leading to the necrosis of hepatocytes (Price and Alberti, 1985).

Materials and Methods

Animals

Fifty Swiss Albino mice weight between 29 to 33 g were obtained Institute of Graduate Studies and Research (IGSR), University of Alexandria during the experimental period these were kept in a well-ventilated animal house and under the control managerial and environmental conditions. These animals were divided in five groups of ten animals each. CCl₄ (1 ml/kg body weight) was administered to groups III and groups IV animals by subcutaneously injection which is well documented to induce acute hepatotoxicity in mice. All animals were made to fast 24 h before the experiment. The duration of the experiment was every other day for three successive weeks. Group I: This group received normal saline (0.9%NaCl) served as negative control. Group II: Animal in this group received subcutaneously olive oil (0.5 ml/kg body weight/day) and served as positive control (vehicle). Group III: Each animal received subcutaneously CCl₄ (1 ml/kg body weight/day) diluted with olive oil (1:1). Group IV: Animals were pretreated with *Spirulina* (800 mg/kg body weight/ 0.5 ml drinking water) orally 30 min after the single injection of CCl₄ (1 ml/kg body weight/day). Group V: *Spirulina platensis* group which received *Spirulina* (800 mg/kg body weight / 0.5 ml drinking water). At the end of experimental period, mice were slightly anaesthetized by diethyl ether (Sigma Chem. Co., St Louis, Mo. U.S.A.)

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Chemicals

All reagents were of the highest purity available. CCl₄ was purchased from Sigma-Aldrich (St. Louis, Mo, USA). *Spirulina* powder was provided from The National Institute of Oceanography and Fisheries (NIOF); Egypt.

Biochemical measurements for the assessment of liver function (Haematological methods)

Haemoglobin (Hb) concentration, red blood cell count (RBC), white blood cell (WBC) and Platelets (PLT) were measured by the method of (Schalm *et al.*, 1975).

Statistical analysis

Data were subjected to one-way analysis of variance applying SAS program (SAS, 2001) using general linear model GLM. Significant differences among treatment means were separated using Duncan's multiple range procedure (Duncan, 1955). The values are expressed as means ± S.E. for 5 rats in each group. *P-Values* < 0.05 were considered significant according to (Snedecor and Cochran, 1982).

Results and Discussion

Carbon tetrachloride (CCl₄) has been commonly used as a hepatotoxin in experimental hepatopathy (Geetha *et al.*, 2008). CCl₄-induced hepatic injury has been extensively used in animal models to evaluate the therapeutic potential of drugs and dietary antioxidants (Hsu *et al.*, 2010). From thousands of years, herbal medicines have been widely used as hepatoprotective and anti-fibrotic drugs in the treatment of liver diseases (Lin *et al.*, 2011). In the present study, the hepatoprotective effect of *Spirulina sp.* has been examined in CCl₄ intoxicated mice. Different biochemical, molecular, cytological and histological parameters were measured.

Complete blood analysis

CCl₄ significantly decreased RBCs counts and Hb concentration. Administration of *Spirulina platensis* significantly increased the RBCs count, Hb and haematocrite concentration when compared to control or carbon tetrachloride- intoxicated group as shown in (Table 1)

Effect on blood platelets

Results indicated that platelet counts (PLT) ×10³/ μl in control group was 230.6±44.88. In CCl₄ -intoxicated mice, platelet counts, was significantly (P=0.0036) decreased in comparison to normal control. Administration of treated group significantly increased platelets counts, to levels nearly similar to that of normal control

Effect on total and differential leucocytic counts

CCl₄ significantly affected total and differential leucocytes counts. There was a significant difference in the total WBCs count between all groups. Lymphocytes and eosinophiles percent were significantly increased by CCl₄ in (group 3). Monocyte percent was significantly increased in treated mice (Group 4) (Table 1)

Table 1 .Effect the *Spirulina platensis* on some hematological indices, WBC and PLT among the five mice groups

hematological Groups parameter	RBCs (×10 ⁶ /L)	PLT (×10 ³ / μl)	WBC (×10 ³ /μl)	HB (g/dl)
Control	4.30±0.03 ^b	216.80±16.72 ^a	8.86±0.07 ^c	13.84±0.22 ^a
Olive Oil	3.99±0.33 ^c	222.90±1.04 ^a	8.94±0.18 ^c	13±0.03 ^a
CCl ₄	2.66±0.93 ^e	141±5.10 ^b	12.74±0.09 ^a	8.70±0.22 ^b
<i>Spirulina</i> + CCl ₄	3.59±0.07 ^d	166.80±33.48 ^b	10.24±0.23 ^b	9.53±1.50 ^b
<i>Spirulina</i>	4.54±0.80 ^a	232.25±1.00 ^a	9.11±0.04 ^c	13.64±0.06 ^a

* RBC: Red blood corpuscles, Hb: Hemoglobin, WBCs: White blood corpuscles, PLT: Platelets. Values are Mean±SD

In the present study, carbon tetrachloride (1 ml/kg body weight) decreased PCV, Hb levels, platelets count and RBCs count. These results corresponded with (Essawy *et al.*, 2010), while this depression in RBCs count and Hb content could be attributed to disturbed hematopoiesis, destruction of erythrocytes, reduction in the rate of their formation and /or their enhanced removal circulation. On the other hand there was a positive effect of the *Spirulina* on the hemopoiesis. Animals in group (5) differ from those in the control one by 5% higher number of red blood. This result supported by (Jensen *et al.*, 2001 & Zhang, *et al.*, 2001 & Simsek, *et al.*, 2007) who found that *Spirulina platensis* has induced significant positive effects on rythropoiesis in adult mice

evidencing by increases of RBC counts and of haemoglobin concentrations. On contrary animals in groups (3) and (4) decreased by 38% and 16% respectively, this means that addition of *Spirulina* has been improved the toxicity of ccl4 by 22 %.

Similar changes were revealed also in the haemoglobin level of *Spirulina* supplemented to ccl4 whereas the level of hemoglobin increased by 17%, from 37% group (3) to 20% group (4). These results were in agreement with (Abdel-Wahhab, et al., 2013) who found that CCl4 significantly affect the quantity and function of Hb molecule whereas total Hb and Oxy-Hb contents decreased significantly concomitant with significant increases in Met-Hb, Sulf-Hb and Carboxy-Hb contents. These changes may, therefore, is a consequence of the increase in oxidative stress which caused by free radicals generated during the metabolic degradation of CCl4. Also Simsek *et al.*, (2009) & Makni *et al.*, (2012) found similar effect of *Spirulina* on total Hb that improved haemoglobine function since they could elevate total Hb and Oxy-Hb contents and decrease Met-Hb formation.

The White Blood Cell (WBC) counts number significantly increased in both treated groups (3) and reached maximal values 12.74 ± 0.21 ($\times 10^3/\mu\text{l}$) ($p < 0.0001$) (Table 1), compared to (1) 8.86 ± 0.15 and (4) 10.23 ± 0.51 respectively. In agreement with that, (Zhang *et al.*, 2001). *Spirulina platensis* is reported to increase the numbers of white cells, nucleated cells, total leukocyte, neutrophil and lymphocyte counts were significantly increased in *Spirulina platensis*- treated rats (300 mg/kg/day) in drinking water for 15th day of treatment and the 30th day.

In comparison to control group (1) Injection of CCl4 increased WBCs count by 43.8%. Moreover group (4) increased by 15.5 %, this may be attributed to the defensive mechanism of immune system as mentioned by (Patrick-Iwuanyanw *et al.*, 2007) so the ability of free radicals to increase WBCs count indicates that these radicals to an extent affected the defense mechanism of treated rats (Oluyemi *et al.*, 2007).

Most reports lend support to a decrease in platelet count associated with the severity of liver injury (Panzer *et al.*, 2006). Effect of platelets on liver regeneration was not addressed till the beginning of the 21st century. There are some reported studies in which platelets were shown to promote liver regeneration (Hesami *et al.*, 2014).

Conclusion

Previous results indicated that immediately following the 21-d period of *Spirulina* administration, in mice blood erythrocyte count and haemoglobin concentration had a tendency to an increase accompanied by a decrease of the mean erythrocyte volume and unchanged blood haematocrit. Leucocyte count also showed an increasing tendency. Treatment with *Spirulina* sp. significantly declined the effects of CCl4 induced damage and it was evidenced by the increase in red, white blood cells and immunity stimulating capacity could be due to the presence of C-phycoyanin in the *Spirulina* alga, which can help build the immunity capacity.

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