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Hepatoprotective Effect of Spirulina Platensis Against Aluminum Chloride Induced Liver Damage in Rats

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Abstract: Aluminum is widely distributed in the environment and is extensively used in daily life throughout the world. It accumulates in all tissues of the mammals and has a significant toxic potential for humans and animal's tissues. The present study was carried out to investigate the possible protective role of spirulina in modulating the hepato-toxicity induced by aluminum chloride in rats. For this purpose, Twenty-eight, male albino rats were divided into four groups (7 rats each); Group (1), received distilled water and served as a control. Group (2) was daily administered spirulina at a dose of 300mg/kg b.wt. by gavage for 30 successive days. Group (3) was daily administered aluminum chloride at a dose of 50 mg /kg b.wt. by gavage for 30 successive days. Group (4) was co-administered aluminum chloride and spirulina with similar doses and duration that mentioned before. At the end of the experimental period, blood samples were collected for hematological studies. Serum samples were separated and used for estimation of hepatic function tests. Liver homogenate was used for oxidative stress biomarkers (lipid peroxidation and antioxidant enzymes; SOD and CAT). The obtained results revealed that co-administration of spirulina with $AlCl_3$ alleviated the hepato-toxic effect of $AlCl_3$ through restoring oxidant-antioxidant balance. It significantly declined the level of lipid peroxidation and increased the activity of antioxidant enzymes (SOD and CAT) in liver. It diminished the oxidative effects of $AlCl_3$ on RBCs membrane and maintained the hematological parameters near normal limits. Moreover, it decreased the elevated activities of liver enzymes (ALT and AST), triglycerides, total cholesterol, LDL-c and bilirubin concentrations and increased the levels of HDL-c, total proteins and albumin. Spirulina is a valuable hepato-protective agent.

Key words: Spirulina · Aluminum Chloride · Hepatotoxicity · Oxidative Stress · Rat

INTRODUCTION

Aluminum (Al) is one of the highly abundant elements that comprise about 8% of the Earth's crust. It is found in combination with oxygen, silicon, fluorine and other elements in the soil, rocks, clays and gems [1]. It is extensively used in medicine as antacid, vaccines, buffered aspirins, phosphate binders and allergen injections [2]. Moreover**,** it is present in many manufactured foods, cheese, tea, cosmetics and is also added to drinking water during purification purposes [3]. Al is still a metal of choice in making various kinds of household cookware and storage utensils throughout the world, especially in developing countries [4].

The extensive use of this metal (food and occupational exposure) was questioned due to its toxic effects. It was included in the priority list of hazardous substances identified by the Agency for Toxic Substances and Disease Registry [5]. It accumulates in various mammalian tissues such as the heart, kidneys, brain and liver and provoked cardiotoxicity, nephrotoxicity, neurotoxicity and hepatic dysfunctions [6]. It has been proposed as an environmental factor that may contribute to some neurodegenerative diseases and affects several enzymes and other biomolecules relevant to Alzheimer's disease [7].

During the last decades considerable attention has been focused on the involvement of oxygen free radical (OFR) in various diseases. Intoxication with Al exacerbates reactive oxygen species (ROS) formation and has been found to cause oxidative damage of lipids, proteins and DNA [8].

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Spirulina platensis (SP) is a unicellular cyanbacterium, belonging to the Oscillatoraceae family that usually grows in the alkaline waters of Africa, Asia, North and South America [9]. It has high nutritional value and wide range of medicinal applications because of its high contents of proteins, carotenoids, vitamins, minerals and various essential fatty acids [10]. Also, Spirulina contains a photosynthetic pigment phycocyanin (PC), which is found to posses antioxidant, anti-inflammatory, neuro-protective, immuno-modulatory and anticancer activities [11].

Nowadays, increased interest in spirulina is based on the fact that, it is believed to be non toxic, bioavailable and provide significant multiorgan protection against many drugs and chemicals induced toxic assaults [12]. Consequently, the aim of the present work is to evaluate the potential hepato-protective effect of spirulina against $AlCl_3$ induced liver damage in rats.

MATERIALS AND METHODS

Animals: Twenty-eight, male albino rats weighing about 100-120 g were used in the present study. They were obtained from the animal house, Faculty of Veterinary Medicine, Cairo University, fed on basal diet and watered *ad-libitum*. Rats were left for two weeks for acclimatization before starting the experiment. All experimental manipulations were undertaken in accordance with the Institutional Guidelines for the Care and Use of Laboratory Animals.

Chemicals

Aluminum Chloride: Aluminum chloride was obtained from ALPHA CHMIKA, India.

Spirulina: *S. platensis* was purchased from DXN marketing BHD, Malaysia in form of tablets.

Experimental Design: Rats were randomly allocated into four groups with seven rats each: Group (1), received distilled water and served as a control. Group (2) was daily administered spirulina at a dose of 300 mg/kg b.wt. by gavage for 30 successive days [13]. Group (3) was daily administered aluminum chloride at a dose of 50 mg/kg b.wt. by gavage for 30 successive days. [14]. Group (4) was co-administered aluminum chloride and spirulina with similar doses and duration that mentioned before.

Collection and Preparation of Samples

Blood Samples: At the end of the experimental period, all rats were fasted overnight. Blood samples were collected from the retro-orbital venous plexus of different rats of each group and were divided into two parts. The first one was anticoagulated by ethylenediamine tetra-acetic acid (EDTA) and used for hematological studies. The second part was collected in clean centrifuge tubes and was allowed to clot, then centrifuged at 3000 rpm for 10 minutes for serum separation. The clear non hemolysed supernatant serum was harvested for biochemical studies. Liver samples were collected from rats of each group for estimation of hepatic oxidative stress.

Preparation of Liver Homogenate: Liver samples from rats of different experimental groups were quickly removed, washed in ice-cold, isotonic saline and blotted individually on ash-free filter paper. The tissues were then homogenized separately in 0.1 M Tris-HCl buffer, pH 7.4. The crude tissue homogenate was then centrifuged at 9,000 rpm for 15 min at 4°C and the supernatant was collected and kept at -40°C until used for estimating lipid peroxidation and the antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT).

Determination of Body and Liver Weights: The body weight of each rat of different experimental group was measured initially at the start of the experiment. At the end of the experimental period, both rats' body and liver weights were measured.

Hepatic Oxidative Stress Parameters

Determination of Lipid Peroxide Levels: Hepatic lipid peroxidation content was evaluated in liver homogenate by measurement of MDA according to Mihara and Uchiyama [15]. In this reaction, thiobarbituric acid (TBA) reacts with malondialdehyde (MDA) in acidic medium at a temperature of 95°C for 30 min with frequent shaking to form thiobarbituric acid reactive substance (TBARs). The absorbance of the resultant pink product is measured at 534 nm.

Determination of Antioxidant Enzymes in Liver Homogenate: The activities of superoxide dismutase (SOD) and catalase (CAT) enzymes in liver homogenate were determined by the method of Kakkar, *et al*. [16] and Aebi [17], respectively. The lipid peroxide levels and antioxidant enzymes activities were done by Bio-diagnostic commercial reagent kits.

Hematological Studies: Hematological parameters including erythrocyte count (RBCs), packed cell volume (PCV), hemoglobin concentration (Hb), total leukocyte count (TLC) and differential leukocyte count (DLC) were done according to Feldman *et al.* [18].

Measurement of Hepatic Function Markers: Serum samples were prepared to assay the following biochemical studies; Alanine (ALT) and aspartate (AST) amino transferases activities were performed according to the Reitman and Frankel [19]. Serum bilirubin was determined after Walter and Gerade [20]. Serum triglycerides, total cholesterol, low density lipoprotein cholesterol (LDL-c) and high density lipoprotein cholesterol (HDL-c) were determined according to Fossati and Principle [21], Allain *et al.* [22], Wieland and Seidel [23] and Burstein [24], respectively. Serum total proteins were determined according to the biuret method after Weichselbaun [25], serum albumin after Dumas and Biggs [26] and serum globulins were determined by subtracting value of serum albumin from the value of serum total proteins. The previous biochemical parameters were assayed using commercial Bio-diagnostic kits.

Statistical Analysis: Data were expressed as means ± SD and were analyzed using one way analysis of variance (ANOVA) within SPSS version 16.0 for windows. Duncan's multiple range test was used to differentiate between means at probability level of ($P<0.05$).

RESULTS

Effect on Body and Liver Weights: Mean values of rats' body weight and liver weights of different experimental groups are illustrated in Table 1. No significant changes were recorded between rats' initial body weights of different experimental groups. Significant decrease was encountered in the final body weight and body gain weight of aluminum chloride administered rats (group 3) in comparable to control or spirulina administered rats. However, Final body weight and body gain weight of rats' concurrently administered spirulina with aluminum chloride were normalized to their control values. Mean values of rats' liver weights showed no significant difference between various experimental groups.

Hepatic Oxidative Stress Parameters: Effect of aluminum chloride and/or spirulina on hepatic malondialdehyde level, SOD and CAT activities are shown in Table 2. The obtained data revealed a significant increase in hepatic MDA levels with a significant drop in the activities of SOD and CAT of aluminum chloride administered rats (group 3) versus control one. Insignificant changes were recorded in the level of hepatic MDA, SOD and CAT activities of spirulina administered group (group2) compared to control group. Co-administration of spirulina with aluminum chloride significantly decreased the values of MDA in comparable to those of aluminum chloride administered group. The activities of SOD and CAT enzymes of rats concurrently administered spirulina with aluminum chloride (group 4) were elevated to match the levels of control group.

Hematological Results: Erythrogram mean values of different experimental groups [packed cell volume (PCV), erythrocytes count (RBCs), hemoglobin concentration (Hb), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC)] are illustrated in Table 3. The obtained results revealed a significant decline in erythrocytic count, PCV%, Hb concentration, MCHC values with a significant elevation in MCV values of $AlCl_3$ administered group (group 3) in comparison to control group. In addition, microscopical examination of the stained blood film revealed the presence of reticulocytosis, poikilocytosis and anisocytosis. Rats' co-administered spirulina with aluminum chloride showed improvement in the previous hematological indices compared to those administered aluminum chloride. Erythrogram mean values of spirulina administered rats were insignificantly changed versus control group.

Means values of *leukogram* (Total leukocytic count, lymphocyte, neutrophil, monocyte and esinophil count) of different experimental groups are illustrated in Table 4. The obtained results showed that TLC and DLC of $AlCl_3$ administered group were not significantly affected when compared with other groups.

Results of Hepatic Function Markers: The activities of serum AST and ALT enzymes, total bilirubin (TB), conjugated bilirubin (BC) and unconjugated bilirubin (BU) are illustrated in Table 5. Significant increases were recorded in the activities of serum amino transferase enzymes (AST and ALT) of $AlCl_3$ administered group (group 3) in comparison to control group. Meanwhile, group of rats' concurrently administered spirulina with $AlCl_3$ showed significant decrease in the elevated activities of these enzymes when compared to $AlCl_3$ group.

Compared to control group, significant elevation was recorded in the values of serum total bilirubin and unconjugated bilirubin of $AlCl_3$ administered group (group 3). However, significant decline was recorded in the previous parameters of rats concurrently administered spirulina with $AlCl_3$ (group 4) compared to those administered $AlCl_3$ alone. Mean values of serum conjugated bilirubin was not significantly change among different experimental groups.

Table 1: Effect of aluminum chloride and/or spirulina on rats' body and Liver weights.

	Parameters			
Groups	Initial body wt. (g)	Final body wt. (g)	Body gain wt. (g)	Liver weight (g)
Group 1 (Control)	114.60±1.67[a]	165.30±5.60[a]	51.00±8.15[a]	7.24±0.65[a]
Group 2 (Spirulina)	115.80±1.48[a]	167.80±6.92[a]	52.00±7.36[a]	7.28±0.50[a]
Group 3 ($Al.Cl_3$)	115.20±3.11[a]	155.30±4.53[b]	40.10±5.18[b]	7.48±0.56[a]
Group 4 (Spirul.+$Al.Cl_3$)	116.60±3.48[a]	163.50±3.74[a]	46.90±4.74[a]	7.70±0.57[a]

Values represent means ± SD. Means with different superscripts (a, b and c) within the same column are significantly different at P <0.05.

Table 2: Effect of aluminum chloride and/or spirulina on hepatic MDA level, SOD and CAT activities of rats

	Parameters		
Groups	Hepatic MDA (µmol/g tissue)	Hepatic SOD (U/mg protein)	Hepatic CAT (U/mg protein)
Group 1 (Control)	39.92±1.71[c]	13.36±1.83[a]	74.80±2.59[a]
Group 2 (Spirulina)	38.76±1.92[c]	14.08±0.54[a]	75.18±3.90[a]
Group 3 ($Al.Cl_3$)	58.2±3.16[a]	8.12±1.21[b]	53.20±3.46[b]
Group 4 (Spirul.+$Al.Cl_3$)	44.4±2.16[b]	12.52±1.12[a]	71.40±3.44[a]

Values represent means ± SD. Means with different superscripts (a, b and c) within the same column are significantly different at P <0.05.

Table 3: Effect of aluminum chloride and/or spirulina on erythrogram of rats

	Parameters				
Groups	PCV (%)	Hb (g/dL)	RBCs ($\times10^6$ /µl)	MCV(fl)	MCHC (%)
Group 1 (Control)	39.8±1.92[a]	13.45±0.93[a]	6.83±0.40[ab]	58.32±1.87[b]	34.04±2.38[a]
Group 2 (Spirulina)	40.6±1.14[a]	14.54±1.19[a]	7.16±0.36[a]	56.77±2.14[b]	35.86± 3.41[a]
Group 3 ($Al.Cl_3$)	34.6±1.34[c]	10.22±1.20[c]	5.28±0.59[c]	65.78±2.24[a]	29.46 ± 4.16[c]
Group 4 (Spirul.+$Al.Cl_3$)	37.4±1.10[b]	11.88±0.95[b]	6.44±0.54[b]	57.81±3.33[b]	31.58 ± 3.19[b]

Values represent means ± SD. Means with different superscripts (a, b and c) within the same column are significantly different at P <0.05.

Table 4: Effect of aluminum chloride and/or spirulina on Leukogram of rats

	Parameters				
Groups	TLC ($\times10^3$/µl)	Lymph. ($\times10^3$/µl)	Neutr. ($\times10^3$/µl)	Monocyte ($\times10^3$/µl)	Esino. ($\times10^3$/µl)
Group 1 (Control)	10.40±1.17[a]	6.52±0.67[a]	3.36±0.38[a]	0.24±0.04[a]	0.32±0.04[a]
Group 2 (Spirulina)	10.64±0.79[a]	6.76± 0.71 [a]	3.56±0.62 [a]	0.26±0.03[a]	0.30±0.02[a]
Group 3 ($Al.Cl_3$)	11.18±0.83[a]	6.38±0.41 [a]	4.12±0.52 [a]	0.27±0.02[a]	0.28±0.06[a]
Group 4 (Spirul.+$Al.Cl_3$)	10.25±2.53[a]	5.98±0.78 [a]	3.72±0.94 [a]	0.23±0.05[a]	0.26±0.08[a]

Values represent means ± SD. Means with different superscripts (a, b and c) within the same column are significantly different at P <0.05

Table 5: Effect of aluminum chloride and/or spirulina on the activity of serum liver enzymes (ALT and AST) and serum bilirubin concentration of rats

	Parameters				
Groups	ALT(IU/L)	AST(IU/L)	TB(mg/dl)	BC(mg/dl)	BU(mg/dl)
Group 1 (Control)	46.80 ± 5.40[c]	72.40 ± 3.21[c]	0.32 ± 0.02[c]	0.11 ± 0.02[a]	0.21 ± 0.03[cb]
Group 2 (Spirulina)	48.40 ± 5.13[c]	70.52 ± 6.52[c]	0.29 ± 0.03[c]	0.10 ± 0.03[a]	0.19 ± 0.02[c]
Group 3 ($Al.Cl_3$)	75.82 ± 5.22[a]	117.2 ± 4.15[a]	0.50 ± 0.10[a]	0.15 ± 0.08[a]	0.35 ± 0.10 [a]
Group 4 (Spirul.+$Al.Cl_3$)	59.82 ± 3.49[b]	89.80 ± 3.49[b]	0.39 ± 0.06[b]	0.14 ± 0.02[a]	0.25 ± 0.02[b]

Values represent means ± SD. Means with different superscripts (a, b and c) within the same column are significantly different at P <0.05.

Table 6: Effect of aluminum chloride and/or spirulina on lipid profile of rats.

Groups	Triglycerides (mg/dl)	T.cholesterol (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)
Group 1 (Control)	65.22 ± 4.09[c]	78.60 ± 3.11[c]	38.72 ± 3.13[b]	29.76 ± 1.73[c]
Group 2 (Spirulina)	64.42 ± 5.18[c]	70.50 ± 2.69[d]	43.60 ± 1.14[a]	27.40 ± 1.52[c]
Group 3 ($Al.Cl_3$)	91.20 ± 4.15[a]	111.0 ± 4.90[a]	30.40 ± 1.67[c]	65.60 ± 3.85[a]
Group 4 (Spirul.+$Al.Cl_3$)	80.60 ± 2.41[b]	90.50 ± 2.69[b]	35.20 ± 3.83[b]	47.40 ± 5.73[b]

Values represent means ± SD. Means with different superscripts (a, b, c and d) within the same column are significantly different at P <0.05.

Table 7: Effect of aluminum chloride and/or spirulina on proteins profile of rats.

	Parameters			
Groups	T. Proteins (g/dl)	Albumin (g/dl)	Globulins (g/dl)	A/G Ratio
Group 1 (Control)	6.64 ± 0.48^{a}	4.24 ± 0.30^{a}	2.40 ± 0.55^{a}	1.87 ± 0.56^{a}
Group 2 (Spirulina)	7.00 ± 0.32^{a}	4.56 ± 0.38^{a}	2.46 ± 0.33^{a}	1.91 ± 0.36^{a}
Group 3 ($Al.Cl_3$)	4.20 ± 0.40^{c}	2.26 ± 0.26^{c}	1.94 ± 0.46^{a}	1.17 ± 0.38^{b}
Group 4 (Spirul.+$Al.Cl_3$)	5.56 ± 0.67^{b}	3.40 ± 0.51^{b}	2.16 ± 0.26^{a}	1.58 ± 0.24^{ab}

Values represent means ± SD. Means with different superscripts (a, b and c) within the same column are significantly different at $P < 0.05$.

Results of lipid profile of different groups are illustrated in Table 6. Statistical analysis of the present data showed that the administration of spirulina significantly declined the level of total cholesterol and elevated HDL-c level in respect to control values. Administration of $AlCl_3$ induced a marked elevation in serum triglycerides, total cholesterol and LDL-c levels with a significant decline in HDL-c level compared to control group. The concomitant administration of spirulina with $AlCl_3$ significantly decreased the elevated triglyceride, total cholesterol and LDL-c levels compared to the levels of $AlCl_3$ administered rats. HDL-c levels were significantly elevated to reach that of control group.

Data of proteins profile (Table 7) showed a significant decrease in the level of serum total proteins, albumin and A/G ratio of $AlCl_3$ administered group. Co-administration of spirulina with aluminum chloride improved the previous values compared to $AlCl_3$ group. Values of serum globulins showed insignificant changes in all experimental groups.

DISCUSSION

Aluminum is widely distributed in the environment and is extensively used in daily life throughout the world. It accumulates in all tissues of the mammals and has a significant toxic potential for humans and animal's tissues [14]. The present study was conducted to investigate the potential protective effects of spirulina against the possible hepatic damage induced by $AlCl_3$ through the evaluation of body weight, lipid peroxidation (LPO) and oxidative stress, hematological indices and serum biochemical alterations in rats.

The obtained results showed that administration of $AlCl_3$ at a dose of 50 mg/kg b.wt. for 30 days resulted in a remarkable decrease in rats' final body weight and body gain weight. The decreased weights may be referred to the effect of $AlCl_3$ on the bioavailability of some nutrients and feed intake by modulation of appetite [27]. However, concurrent administration of spirulina with $AlCl_3$ restored rats' body weights and body gain towards normal. Spirulina contains proteins, carbohydrates, essential fatty acids, vitamins, minerals, carotenes, chlorophyll *a* and phycocyanin. It is an ideal food and dietary supplement for the 21st century by the Food and Agriculture Organization (FAO) of the United Nations [28].

Oxidative stress is known to play a key causative role in many diseases including liver damage [29]. In the view of the present data, $AlCl_3$ induced a status of oxidant/antioxidant imbalance as indicated by increased hepatic MDA level with a concomitant depletion in the activities of SOD and CAT enzymes in the liver tissue. MDA is a marker for lipid peroxidation (LPO) that considered as the main manifestations of oxidative damage [30]. Several investigations reported that $AlCl_3$ has the ability to potentiate iron-mediated LPO [31, 32]. Disruption in mineral balance through replacing iron ions with Al and the subsequent increase in the amount of the free iron can explain the increased LPO. The free iron ions have a strong catalytic power to generate highly reactive hydroxyl radicals from hydrogen peroxide through Fenton's reaction [33]. The body has anti-oxidative mechanisms to stabilize oxidative molecules, control lipid oxidation and keep these radicals in balance. When free radicals are generated, the body defends itself from these radicals by endogenous antioxidants [34]. Administration of $AlCl_3$ decreased the activity of antioxidant enzymes (SOD and CAT) in the liver tissue. The reduction in the activity of SOD and CAT reflect the reduced synthesis of these enzymes due to higher intracellular concentrations of Al and/or accumulation of free radicals. Further, the inhibition of the activities of these enzymes may also be referred to the effect of Al in declining the expression of mRNA of endogenous antioxidants [35]. Nevertheless, simultaneous administration of spirulina with $AlCl_3$ restored the oxidant/antioxidant balance as reflected by the decrease in MDA level and the stimulation of the antioxidants enzymes (SOD and CAT) in the liver. The antioxidant effect of spirulina referred to its active components which provokes free radical scavenging activities [36].

Concerning hematological indices, significant decrease in erythrocytic count, PCV% and Hb concentration was recorded in $AlCl_3$ administered rats compared to control one. The previous changes were coupled with increased MCV and decreased MCHC which suggest developing of macrocytic hypochromic anemia. Several mechanisms have been implicated in aluminum-induced anemia. It may be due to a shortened life span of circulating erythrocytes and reduced RBCS production in bone marrow. $AlCl_3$ increases the production of free radicals and decreases the erythrocyte ATP concentration resulting in increased membrane fragility and increased RBCs destructions [14]. Furthermore, $AlCl_3$ inhibits heme synthesis, either by inhibition of enzyme activity or interference with iron incorporation or utilization [37]. The presence of reticulocytosis, poikilocytosis and anisocytosis in the blood smear reflected the bone marrow erythroid hyperplasia and regenerative anemia. The previous indices are recouped considerably in rats' concurrently administered spirulina with $AlCl_3$ compared with group of rats administered $AlCl_3$. The antioxidants effects of spirulina have been instrumental in avoiding oxidative effects of $AlCl_3$ on RBCs membrane [38] and consequently maintain the hematological parameters near normal limits. In addition, Morcos *et al*. [39] found that phycocyanin (the major pigment constituents of spirulina) stimulates the secretion of erythropoietin and regulates bone marrow stem cell production of red blood cells.

Regarding serum hepatic function markers, the activities of serum ALT and AST were significantly increased in $AlCl_3$ administered group. The increased activities of these enzymes suggest hepatic damage induced by $AlCl_3$. The obtained data are in agreement with the earlier works of Chinoy and Memon [40] and El-Demerdash [41]. They found that exposure to $AlCl_3$ caused necrosis to the liver with the subsequent release of these enzymes from the injured hepatic cells to the plasma.

The recorded hyperbilirubinemia with increased level of unconjugated bilirubin of $AlCl_3$ administered group may be resulted from increased bilirubin production as sequence to the destructive effect of $AlCl_3$ on erythrocyte and this is in accordance with the present results. The obtained results coincide with the previous study of Yousef *et al*. [42]. Moreover, Mangood *et al*. [7] found that the induction rate of serum bilirubin was associated with free radical production.

In view of the current data, $AlCl_3$ administered group showed a significant increase in serum triglycerides, total cholesterol and LDL-c with a significant decrease in HDL-c compared to control group. Accumulation of $AlCl_3$ in the liver resulted in increased LPO and loss of membrane integrity which might be important determinants of altered lipid metabolism [42]. On the contrary, administration of spirulina with $AlCl_3$ significantly decreased the elevated triglyceride, total cholesterol and LDL-c levels compared to the levels of $AlCl_3$ administered rats. This anti-hyperlipidimic effect of spirulina may be primarily attributed to its antioxidant activity and the protection of cellular membrane integrity from Al-induced oxidative damage [43]. Furthermore, another study by Cheong *et al*. [44] indicated that phycocyanin of spirulina diminished the intestinal absorption of cholesterol as well as the re-absorption of bile acids in the ileum.

The inhibitory effect of $AlCl_3$ on protein profile is in agreement with the finding of El-Demerdash [41]. The recorded hypoproteinemia with hypoalbuminemia may be attributed to higher intracellular concentration of Al in the liver which could be resulted in reduced protein synthesis [45]. Free radicals may also be implicated in the observed decline in protein content since exposure to the free radicals leads to protein fragmentation, protein peroxides generation, enzymatic oxidation and degradation of proteins [46].

Co-administration of spirulina with $AlCl_3$ improved the previous serum biochemical parameters. There was a remarkable decrease in the activities of ALT and AST, triglycerides, total cholesterol, LDL-c and bilirubin concentrations with increased levels of HDL-c, total proteins and albumin. The hepato-protective effect of spirulina may be referred to its active components; B-carotene, blue pigment phycocyanin, linolenic acid, sulfated polysaccharide, vitamins (C and E) and selenium which provoke the activity of free radical scavenging enzyme system that render hepatic protection [47].

Indeed, it could be concluded that oral administration of spirulina at a dose of 300 mg /kg b.wt. minimized the hepatotoxic effect of $AlCl_3$ which may be due to the role of spirulina as antioxidant agent. It significantly declined the level of hepatic lipid peroxidation and increased the activities of antioxidant enzymes (SOD and CAT) which in turn were reflected by improvement in the hepatic function. Therefore supplementation with spirulina may be useful as a hepatoprotective therapy in cases of intoxication with aluminum.

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