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Original Research Article

In vitro and In vivo antioxidant effect of *Spirulina platensis* against Lead induced toxicity in rats

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Abstract

Lead is considered one of the wide spread environmental pollutants in Iraq. Nowadays, some types of algae can be the solution by utilizing them as natural medications to cure many diseases. This scientific article is intended to examine *spirulina* action as an antioxidant to cure lead acetate induced injury in rats. The total numbers of rats used were (48), the rats were divided into eight groups, (42 rats) represented the treated group and 6 rats represented the control group. 36 Rats of treated groups were injected initially with different concentrations of lead acetate; while the rest 6 rats were only fed with *Spirulina*. All our samples were examined by biochemical, hematological and immunohistological methods. Our experiments proved that *Spirulina* had an antioxidant action which can support the body defense system. Malondialdehyde (MAD), Superoxide Dismutase (SOD) and Catalase (CAT) were increased in lead injected group; while they decreased in the *spirulina* fed group. There was a significant enhancement in lipid profile values of treated group that were fed *spirulina*. In addition, it noticed that the values of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in rats injected by lead, then fed *spirulina* were decreased; this decrement was evidence in the histological results. Moreover, the hematological results of rats fed with *spirulina* appeared that the leukocyte and platelet numbers also decreased; while the erythrocyte, hemoglobin, and hematocrit levels were increased, unlike, rats injected with only lead.

The conclusion was that *Spirulina* ability to do obvious decrement in the poisonous action of lead was done by its scavenger free radical activity and its effective antioxidant activity.

Keywords: In vivo, Antioxidant, Spirulina, Lead toxicity, Hepatoprotective

Introduction

Lead is a toxic metal and considered as widespread environmental pollutant that induced many of behavioral, biochemical and physiological effects in humans. Conversely, alga is found to be a natural therapy that recently drew attention due to it is limited side effects. The blue-green algae (Cyanophycophyta or cyanobacteria) are prokaryotic (nucleus-lacking), almost unicellular algae; their cell walls show some chemical similarity to those of bacteria. *Spirulina* (filamentous microscopic cyanobacteria) is non-

toxigenic spiral alga (Teneva et al., 2005). It is found in the alkaline water with elevated pH and high temperature within 30-35 C° (Colla et al., 2007). *Spirulina* has been reported to be the best food for the future because of nutritional high value such as proteins, vitamins, minerals and an essential fatty acid (Ravi et al., 2010). Some species of *Spirulina* have been classified as a new record in Iraq, such as *Arthrospria jenner* by Al-yassiry (2014), that ensured as new recorded according to Checklist of Algal Flora in Iraq (Maulood et al., 2013).

The blue-green alga *S. platensis* has cell walls formed from soft mucopolysaccharides, make it easily digested by the body. Mainly, it formed from 55-70% protein, carbohydrates 15-25%; lipids 6-13%; Polyunsaturated fatty acids (PUFAs) constitute 1.5-2% of the total lipid content of this alga, and it is rich in Gamma-linolenic acid (36% from the total PUFAs); plus vitamins and minerals 2.2-4.8 (Belay, 2002). *Spirulina platensis* attracted attention not only in the food aspects but also the progress of potential pharmaceuticals.

Lead is one of the heavy metals that was intensively studied, there is no safe blood level of it and in most cases lead poisoning has no symptoms. When lead enters the body will cause free radical generation, then starts serial reactions that result in lipid peroxidation (another biomarker of oxidative stress) by disrupting of cell membranes; protein oxidation (altering the function) and oxidation of nucleic acids like deoxyribonucleic acid and ribonucleic acid which leading to mutation or cancer (Gurer and Ercal, 2000). Lead also creates hemoglobin oxidation, which leading to RBC hemolysis (Patrick, 2006).

Free radicals defined as atoms which contain at minimum one unpaired electron in the outer orbit. Although free radicals play an effective role in biological systems, they may result in extreme damage to cell molecules when present in extreme amounts. Although the body cells are continually prone to ROS attack (Reactive oxygen species); there is a balance between production and scavenging of ROS molecules (Gupta, 2010). Free radicals can cause damage to parts of cells, such as proteins, DNA, and cell membranes by stealing their electrons through an operation called oxidation.

Antioxidants are substances found in low concentrations in the body, its role is to cause retarding or inhibiting oxidative processes, and at the same time it is susceptible to oxidation. The antioxidant as body defense systems are usually being endogenous, like superoxide dismutase SOD, glutathione peroxidase, catalase); and exogenous which come from the food like flavonoids, carotenoids, vitamin E, vitamin C; also unsaturated fatty acids UFAs (Hosseini et al., 2013).

Free radicals may cause heart diseases, cancer, and weak immune system (Valko et al., 2007). Under the influence of lead, two different routes will be triggered; first one is generation of ROS, second, depletion of the antioxidant reservoir such as (SOD, GSH, GPX, CAT) (Flora et al., 2002). Malondialdehyde (MDA) is a good marker for damage due to the effect of free radical and oxidative stress; it is a suitable biomarker for lipid peroxidation (Dauqan et al., 2011).

The objective of this study was to assess the protection action of *Spirulina platensis* extract against the adverse effect of lead acetate. Our study deal with the biochemical, hematological and histopathological alters in male and female rats.

Materials and Methods

Source of S. *platensis*

The strain of *S. platensis* was isolated from freshly collected water from the Tigris River in Baghdad-Iraq, located in the center of Baghdad on longitude 44°22'42.24'E and latitude 33°20'30.88' N.

Aqueous extraction method

125 grams of dried *S. platensis* aqueous extract powder were suspended in 1000 ml of distilled water, and then shaken continuously for 24 hours at 30 °C. The mixed substances were centrifugation at 5000 rpm for 10 minutes and filtered by Whatman filter paper No.1 to remove the cell debris. The extract was evaporated by using rotary evaporator at 35 °C and 60 rpm and stored at 4°C before use for the experiments (Chu et al., 2010).

Detection of some vitamins, fatty acids, and essential minerals

HPLC Chemical Analysis: Qualitative and quantitative analyses of *S. platensis* aqueous extract were used by High-Performance Liquid Chromatography HPLC/ESI-MS (Mahakhart et al., 1998).

Atomic Absorption Spectrophotometer: Flame and flameless atomic absorption spectrometry were applied to a determination of Iron, Zinc, Calcium and

Selenium concentrations in the *S. platensis* aqueous extract according to Sawidis et al. (2001).

Experimental design

Animals: Rats were obtained from Central Public Health Laboratory (Iraqi Ministry of Health). Animal handling and all procedures on animals were applied according to the guidelines of the Animal Ethics Committee, Baghdad University, Iraq.

In vitro and In vivo antioxidant test: The free radical scavenging action of algal extracts were measured by the DPPH assay (Kumar et al., 2008). Several concentrations were mixed (25, 50, 75, 100, 250, 500 and 1000 mg/ml).

While in vivo antioxidant capacity of *S. platensis* was measured by evaluated some physiological and histopathological effects of albino rats after 40 days. Lead acetate was chosen as a generator of free radicals in the bodies of rats. Forty-eight rats weighting 135 ±5 g were used in this study. The animals were caged in a temperature (25 ± 1 °C), humidity controlled room and a 12-h light-dark cycle.

There was free space for rats to get tap water and forage (standard pellet). The animals were divided into 8 groups as a complete randomized design, each group contain six animals, and were given a uniform volume 0.5 ml injection of lead acetate, and 1 ml orally of *Spirulina* extract. All animals had received humane care in compliance according to the guidelines of the animal Care. The groups were divided as follows:

Group 1: (Control) Rats took taps water and ate standard pellet only.

Group 2: (*Spirulina*) Rats were given 1000 mg/kg of *Spirulina* extract orally.

Group 3: (Pb 30) Rats were injected with 30 mg/kg of lead-acetate, daily for 40 days.

Group 4: (Pb 30 + *Spirulina*) Rats were injected with 30 mg/kg of lead-acetate + orally 1000 mg/kg of *Spirulina* extract by stomach tube once/ day for 40 days.

Group 5: (Pb 60) Rats were injected with 60 mg/kg of lead-acetate, daily for 40 days.

Group 6: (Pb 60 + *Spirulina*) Rats were injected with 60 mg/kg of lead-acetate + orally 1000 mg/kg *Spirulina* extract by stomach tube once/ day for 40 days.

Group 7: (Pb 90) Rats were injected with 90 mg/kg of lead-acetate, daily for 40 days.

Group 8: (Pb 90 + *Spirulina*) Rats were injected with 90 mg/kg of lead-acetate + orally 1000 mg/kg *Spirulina* extract by stomach tube once/day for 40 days.

At the end of the experience period, the rats were fasted overnight and anesthetized to obtain 3.0-3.5 ml of blood sample withdrawn by cardiac puncture, and this blood was divided to 2 parts. One of them was centrifuged at 3000 rpm to obtain the serum which was kept frozen at -20 °C until used for analysis, and the remaining part of blood were used for hematological parameters. After that animals had dissected (according to the guidelines of the Animal Ethics Committee, University of Baghdad, Iraq) liver was cutted from all rats and prepared for histological study.

Parameter measurement

Lipid Profile Measurement (TC, TG, HDL, LDL, and VLDL): were estimated by using the procedure of commercially available kit (Spinreact Spain).

Liver function enzymes: AST and ALT were measured by using the procedure of kits provided by Biomerieux-France for the colorimetric determination in serum.

Enzymatic antioxidants (SOD, MDA, CAT): were measured as described by (Gao et al., 1998; Guidet and Shah, 1989) respectively. While Catalase activity (Cat) was measured by using the procedure of Biovision-USA kits at 750 nm.

Hematological measurements: Complete blood counts (CBC) were done by an automated digital counter.

Histopathological studies: Liver was gathered from each group and washed with normal saline, and kept in 10 % formalin solution for fixation, then processed routinely as described by (Bancroft, 2008).

Statistical analysis: The results were revealed as Mean± SE (standard error of the mean). Statistical analysis was accomplished by analysis of variance (one-way ANOVA) with the least significant difference (LSD) test as an indication of significant effect compared with the control group. All calculations were performed using the statistical analysis system program (SAS, 2012).

Results

Active ingredients of *Spirulina platensis*
The results of HPLC analysis as illustrated in table 1, indicate that 1 g of *Spirulina* contains high quantity of vitamins A, C, E, and Gamma-linolenic acid. As well as it contains high levels of trace elements especially iron and calcium.

Antioxidative Capacity (Free radical scavenging ability)
In vitro antioxidant results
The results of the anti-radical action of *S. platensis* aqueous extract which had measured by DPPH showed that color turns from purple to yellow at all seven concentrations. It was observed that 1000 mg/ml was significantly better than the other concentrations by achieving the higher antiradical activity 91.362%; as shown in table 2. So it is the elected concentration which was given to the laboratory animals in the in vivo antioxidant experiment.

In vivo antioxidant results (Laboratory animals experiment)
Spirulina is generally considered safe for consumption by human, based on its long history of using it as a food source. It was obvious that ingestion of 1g/kg of *Spirulina* for 40 days had no effects on behavior, pellet and water intake, growth rate, and survival. Hematologic and clinical chemistry analyses showed no abnormality behaviors. In addition, no microscopic changes were examined with histological evaluation.

Lipid profile and Liver function enzymes
The changes in the levels of sera lipids in the control and all experimental groups are illustrated in table 3, with the results of liver function enzymes AST and ALT. The same table views that *Spirulina* increase HDL and decrease cholesterol, TG, LDL and VLDL, and decreases the high levels of enzymes AST, ALT due to lead poisoning, as compared with control group.

Antioxidant enzymes (MDA, SOD, and CAT)
The results of serum endogenous antioxidant enzymes represented in table 4 showed that *Spirulina* extract dramatically inhibit the production of malondialdehyde (MDA), SOD and CAT, indicating that *Spirulina* has potent antioxidant action.

Hematological results - Complete blood counts (CBC)
Hematological parameters in table 5 show significant increment of WBC count in lead injected groups 30, 60, 90 mg/kg, compared with control and *Spirulina* fed group. Also, there was a significant reduction of RBC count, Hb and PCV in lead injected groups compared with increment of these parameters in *Spirulina* fed group.

Histopathological results
The histopathological alters in rats liver sections were observed at all groups. The adding figures demonstrate the changes of two doses 30 and 60 mg/kg of lead acetate. Fig. 1 represents rats injected with 30 mg/kg of lead acetate and it shows fatty degeneration, necrosis of hepatocytes, nuclear pyknosis and proliferation of kupffer cells; compared with fig. 2 which belongs to rats injected with 30 mg/kg of lead acetate + 1000 mg/kg b.w. of *Spirulina* extract showing necrosis of hepatocytes with infiltration of mononuclear leukocytes.
While in fig. 3; a dose of 60 mg/kg lead acetate shows acute changes that characterized by cellular swelling, necrosis, pyknosis and Karyorrhexis; compared with fig. 4 belongs to rats injected with 60 mg/kg of lead acetate + 1000 mg/kg b.w. of *Spirulina* extract showing sinusoidal congestion and hypercellulartiy.

Discussion

The results exposed that the quantity of micronutrients produced by S. platensis matching with the antioxidant potential. The vitamins A, E, and C were considered as non-enzymatic antioxidant that acts as membrane antioxidants. β-carotene protected against oxygen-mediated lipid peroxidation (Schafer et al., 2002). Polyunsaturated fatty acids have 18 carbon atoms, in particular, the omega-6 (ω-6), the results were identical with Pierlovisi (2007) considering that *S. platensis* is one of the best mines of gamma-linolenic acid ranked after human milk and some high expense vegetable oils.
Although the body can synthesis most of the fats it needs from the diet, but two essential fatty acids linoleic and linolenic acid cannot be synthesized in the body and must get from food, called Omega-3 and omega-6 fatty acids which are important in the ordinary functioning of all tissues of the body (Groff et al., 1995).

All *Spirulina* nutrients, mainly calcium and iron made it suitable nutritional supplement for anyone suffering from anemia or osteoporosis caused by lack of calcium, especially that absorption of *S. platensis* iron is 60% more than ferrous sulfate presented in iron supplements (Johnson and Shubert, 1986). And for selenium, beside its other functions it can be act as radical scavenging (Huang et al., 2007). As well as zinc element competes with iron and copper inside the cell membrane, inhibits the NADPH-oxidase enzyme, and reduces chronic inflammation and hyperglycemia. Therefore, it can act as a protecting agent against oxidative stress (Marreiro et al., 2017).

The DPPH radical scavenging activity is one of the most frequently methods used for scanning the antioxidant activity of any extracts. Our data in DPPH assay revealed that the scavenging action boost with increasing concentration of *S. plantensis* extract. The highest scavenging action of *Spirulina* observed in line with Sahu *et al.* (2013).

Environmental exposure to the toxic levels of lead is a common health problem due to its frequency distribution in the environment. It has competition with essential metallic ions for binding sites, inhibiting enzyme activity, or altering the transport of essential ions such as calcium (Flora et al., 2007).

Lipid profile parameters: It's well known that HDL-C has protective ability against the progress of atherosclerosis and many other diseases. The increasing of HDL level and decreasing of Cholesterol, TG, LDL, and VLDL in the *Spirulina* feed group may be happened due to the active ingredients of *Spirulina* which responsible for the hypolipidemic activity. The cholesterol decrease effect of statins by inhibition of the enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA reductase). The omega-6 fatty acids (GLA) in S. *platensis* will balk the accumulation of cholesterol in the body (Samuels et al., 2002). This may partly interpret the decrease harmful effects of blood lipids.

Liver functional enzymes: The liver is the most sensitive organ for peroxidative damage. Rats which given *Spirulina* plus lead acetate revealed a significant decreases of elevated liver enzymes levels AST and ALT which is an important tool of the biomarker in the diagnosis of hepatic damage because they conducted with the circulation after cellular damage (Dong et al., 2009).

The results indicate *Spirulina* hepatoprotective effect and improvement in the oxidative stress of liver cells. Any elevation of liver enzyme resulted from leakage of liver damage cells and from the disturbance and dysfunctions in liver function enzymes (Abu Zeid, 2001).

Antioxidants enzymes role: The levels of MDA strongly correlate with a lead concentration that indicates oxidative stress, lipid peroxides are known to be harmful to cells and tissues (Linden et al., 2008). Conversely, there was a significant reduction in the elevated levels of blood MDA, SOD and CAT in the *Spirulina* fed rats, which indicate that *Spirulina* reduces the oxidative stress via its antioxidant components like vitamin E and C, and Gamma-Linolenic Acid and minerals (Karadeniz et al., 2008). Antioxidant enzymes such as SOD (a Cu^{2+} dependent enzyme) and Catalase play important role in the intracellular defense against oxygen radical damage to aerobic cells (Hemalatha et al., 2012).

Hematological parameters: The results showed a significant elevation in total leukocyte count (WBC) in rats treated with lead acetate, the WBC is part of the immune system response; protect the body from bacteria and infection (Erlinger et al., 2004). Rats which given *Spirulina* plus lead acetate exposed a significant improvement of elevated WBC.

Lead can reduce the lifespan of circulating erythrocytes by increasing the fragility of cell membranes (Annabi and Nehde, 2007). Significant reduction in RBC, Hb resulted by accumulation of metal inside the red blood cell and perhaps inhibition ferrochelatase, an enzyme which responsible for linking iron to the globin protein, this drops refer to anemia (Al-Hamdany, 2010).

Lead has inhibitory effects on three key enzymes implicated in the synthesis of heme- δ - aminolevulinic acid dehydratase (ALAD), aminolevulinic acid synthetase (ALAS) and ferrochelatase (Ashour et al., 2007). It was observed a good hematological potential of *Spirulina* by the increase in RBC, Hb and PCV values. This can explain a high content of iron in *Spirulina* extract.

As for platelet count, oxidative stress is cause of abnormal platelet function. Antioxidant enzymes SOD and CAT are the defense mechanisms in erythrocytes, also small increases in a Ca^{+2} lead to platelet activation, so maintain stable calcium is important to

keep platelets in a resting state (Bergmeier and Stefanini, 2009).

The hepatic histological changes, which were observed in the liver of lead acetate intoxicated rats contributed to increased levels of lipid peroxidation. There was a relation between the hepatic tissue damage and elevation in the liver enzymes AST and ALT, due to lipid peroxidation of cell membranes that create leakage of cellular components, this harmful effects returning to the toxicity of lead against the liver and the body as a whole. Increment in AST and ALT (as a cytosolic creator enzymes) reflecting hepatocellular necrosis (Urmila et al., 2012). And this necrosis was improved in the hepatic tissue sections in the *Spirulina* fed group to return to free radical-scavenging activity.

After all that, we can notice from this study of in vitro and in vivo there was a proved antioxidant action of *S. platensis* extract. It was concluded that the feeding of *Spirulina* at a dose of 1 g/kg significantly decreased the toxic effects of lead, by enhances the body defense system through scavenging the free radicals and improving the effectiveness of endogenous antioxidants. In addition to hypolipidemic ability of *S. platensis* which indicate a protective action in the cardiovascular system. As well as improvement of hematological parameters indicate the ability to treat anemia.

Table - 1: Ingredients found in the S. *platensis* aqueous extract

Micronutrients in *S. platensis*	**Nutritional value per mg/g**	**Nutrients %**	**Required adult daily dose mg/day**
Vitamin A	18.1	1.81 %	0.9 **
Vitamin C	3.91	0.39 %	90 *
Vitamin E	17.2	1.72 %	15 *
Gamma linolenic acid Omega-6	29.1	2.91 %	-
Iron element	21	2.1 %	8 male 18 female **
Zinc element	1.2	0.12 %	15 **
Calcium element	26	2.6 %	1100 ***
Selenium element	0.5	0.05 %	0.05 *

* Institute of Medicine, 2001
** Ross et al., 2011
*** Groff *et al*., 1995

Table - 2: DPPH free radical scavenging activity on different concentrations of the *Spirulina platensis* extract

Extract concentrations µg/ml	**Mean ± S.E Absorbance 517 nm**	**Anti-radical activity (%)**
DPPH (control)	0.301 ± 0.002	0
25	0.154 ± 0.002	48.837
50	0.132 ± 0.002	56.146
75	0.116 ± 0.003	61.461
125	0.091 ± 0.002	69.767
250	0.072 ± 0.002	76.079
500	0.043 ± 0.003	85.714
1000	0.026±0.002	91.362

Table - 3: Effect of *S. platensis* aqueous extract on lipid profile and liver enzymes in rats treated with lead-acetate

Group	Mean ± SE						
	Cholesterol mg\dl	**Triglyceride mg\dl**	**HDL mg\dl**	**LDL mg\dl**	**VLDL mg\dl**	**GOT (AST) U/L**	**GPT (ALT) U/L**
Control	77.67 ±2.03 ef	59.00 ±2.08 d	12.70 ±0.51 b	53.17 ±1.76 cd	11.80 ±0.42 e	72.66 ± 1.76 f	22.67 ±0.67 d
Spirulina mg\kg	74.67 ±2.60 f	57.67 ±1.85 d	16.20 ±0.95 a	46.93 ±3.84 d	11.53 ±0.37 e	68.33 ± 1.20 f	20.33 ± 0.33 d
Pb 30 mg\kg	88.33 ± 2.03 cd	92.00 ±1.73 c	12.10 ±0.37 b	57.83 ±1.82 c	18.40 ±0.34 cd	80.67 ±1.20 e	23.33 ±2.84 d
Pb 30 + *Spirulina* mg\kg	84.33 ±1.45 de	87.67 ±2.96 c	13.06 ±0.72 b	53.73 ±2.05 cd	17.53 ±0.59 d	70.00 ±3.60 f	21.33 ±1.20 d
Pb 60 mg\kg	94.67 ±1.20 c	122.67 ±2.91 a	11.83 ±0.35 b	58.30 ±1.17 c	24.53 ±0.58 b	148.00 ±3.51 b	30.33 ±1.45 bc
Pb 60 + *Spirulina* mg\kg	84.33 ±2.73 de	104.67 ±5.48 b	11.93 ±0.26 b	51.46 ±3.32 cd	20.93 ±1.09 c	113.33 ±0.88 d	25.33 ±2.03 cd
Pb 90 mg\kg	159.33 ±2.33 a	122.67 ±2.91 a	11.53 ±0.32 b	112.40 ±2.23 a	35.40 ±0.87 a	168.00 ±3.78 a	40.83 ±2.20 a
Pb 90 + *Spirulina* mg\kg	136.67 ±2.90 b	120.33 ±8.41 a	16.97 ±0.59 a	95.63 ±2.42 B	24.07 ±1.68 B	124.00 ±3.05 c	30.67 ±1.45 b
LSD value	**6.694 ***	**12.422 ***	**1.670 ***	**7.496 ***	**2.578 ***	**7.916**	**5.117**

Means having with the different letters in same the column differed significantly.

Table - 4: Effect of *S. platensis* aqueous extract on Antioxidant enzyme (SOD, MDA and CAT) in rats treated with lead acetate

Groups (mg\kg)	Mean ± SE		
	SOD (U/ml)	**CAT (U/ml)**	**MDA (U/ml)**
Control (without treatment)	2.50 ± 0.06 a	1.80 ± 0.21 ab	1.70 ± 0.23 bc
Spirulina 1000	1.53 ± 0.14 b	0.967 ± 0.06 d	1.03 ± 0.09 cde
Pb 30	1.90 ±0.11 ab	1.40 ±0.11 bcd	1.63 ±0.19 cd
Pb 30 + *Spirulina*	1.87 ±0.14 ab	0.990 ±0.11 cd	0.676 ±0.03 e
Pb 60	2.50 ±0.30 a	1.87 ±0.12 ab	2.43 ±0.29 b
Pb 60 + *Spirulina*	1.40 ±0.75 ab	1.77 ±0.29 abc	1.10 ±0.53 cde
Pb 90	2.50 ±0.31 a	2.30 ±0.34 a	3.33 ±0.23 a
Pb 90 + *Spirulina*	1.50 ±0.15 b	2.00 ±0.49 ab	0.900 ±0.17 de
LSD value	**0.965 ***	**0.778 ***	**0.789 ****

Means having with the different letters in same the column differed significantly.

Table - 5: Effect of *S. platensis* aqueous extract on WBC, RBC, Hb, PCV and Platelets in rats treated with lead acetate

Mean ± SE					
Group	**WB Cell x10^3**	**RBC Cell x10^6**	**Hb mg\dl**	**PCV %**	**Platelets Cell x10^3**
Control	8.20 ±2.13 ef	5.76 ±0.35 bc	10.73 ±1.21 bc	31.80 ±3.90 bc	741.00 ±3.78 f
Spirulina mg\kg	7.43 ±0.97 f	7.70 ±0.86 a	15.20 ±1.05 a	42.30 ±3.90 a	870.33 ±5.17 e
Pb 30 mg\kg	20.26 ±3.00 cd	4.72 ±0.76 cd	9.50 ±0.49 bcd	30.53 ±1.87 bc	1308.0 ±6.11 b
Pb 30 + *Spirulina* mg\kg	16.67 ±0.69 de	7.23 ±0.07 ab	11.67 ±0.32 b	33.03 ±0.89 b	1270.00 ±4.72 c
Pb 60 mg\kg	20.26 ±3.00 b	4.58 ±0.49 cde	6.60 ±1.15 de	24.87 ±2.46 cd	1326.33 ±2.60 a
Pb 60 + *Spirulina* mg\kg	15.43 ±2.52 de	4.02 ±0.03 de	5.07 ±0.76 e	28.63 ±0.77 d	1118.00 ±3.21 d
Pb 90 mg\kg	59.67 ±2.60 a	3.31 ±0.25 E	5.16 ±0.84 e	21.06 ±2.95 cd	1307.33 ±4.17 b
Pb 90 + *Spirulina* mg\kg	27.33 ±1.45 c	6.07 ±0.94 abc	8.16 ±1.47 cd	30.33 ±2.77 bc	1315.33 ±3.75 ab
LSD value	**7.244 ****	**1.726 ****	**2.952 ****	**8.061 ****	**12.95 ****

Means having with the different letters in same the column differed significantly.

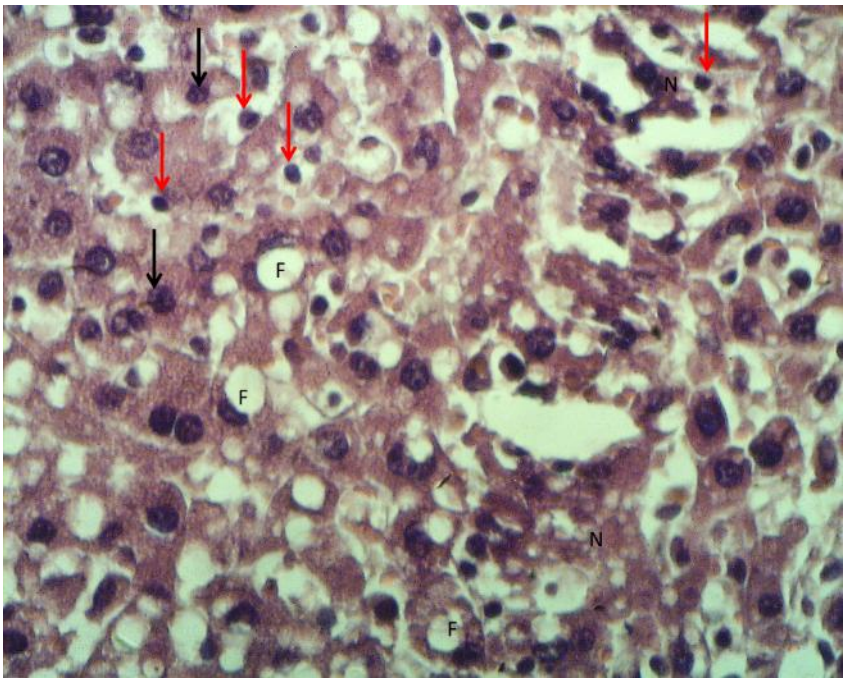

Fig - 1: Magnified section of liver for rats injected with lead acetate a dose of 30 mg/kg shows fatty degeneration (F) with necrosis of hepatocytes (N), nuclear pyknosis (black arrows) and proliferation of kupffer cells. (H&E 400X)

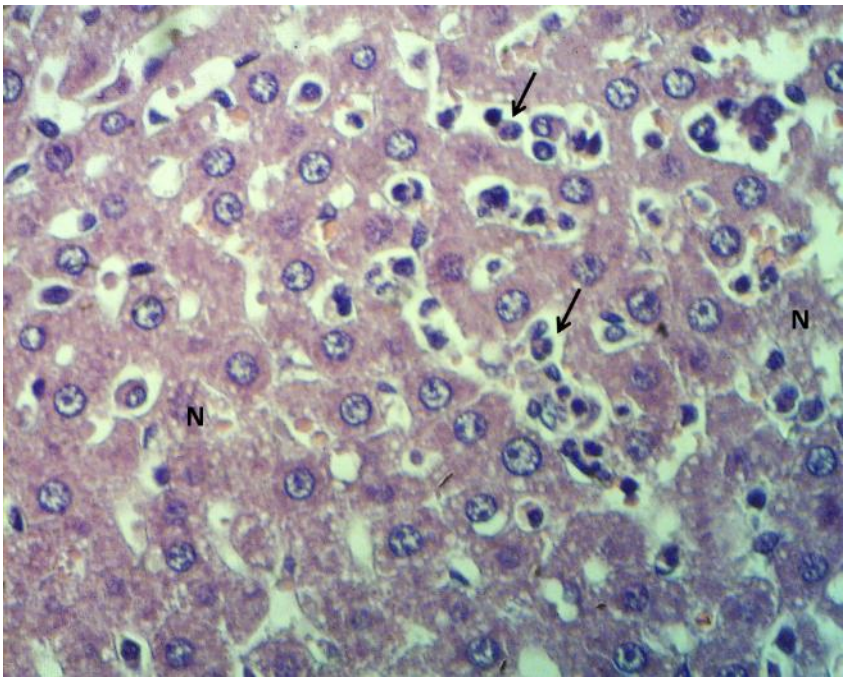

Fig - 2: Magnified section of liver for rats injected with lead acetate a dose of 30 mg/kg + orally 1000 mg/kg *Spirulina* extract shows necrosis of hepatocytes (N) with infiltration of mononuclear leukocytes (arrows). (H&E 400X).

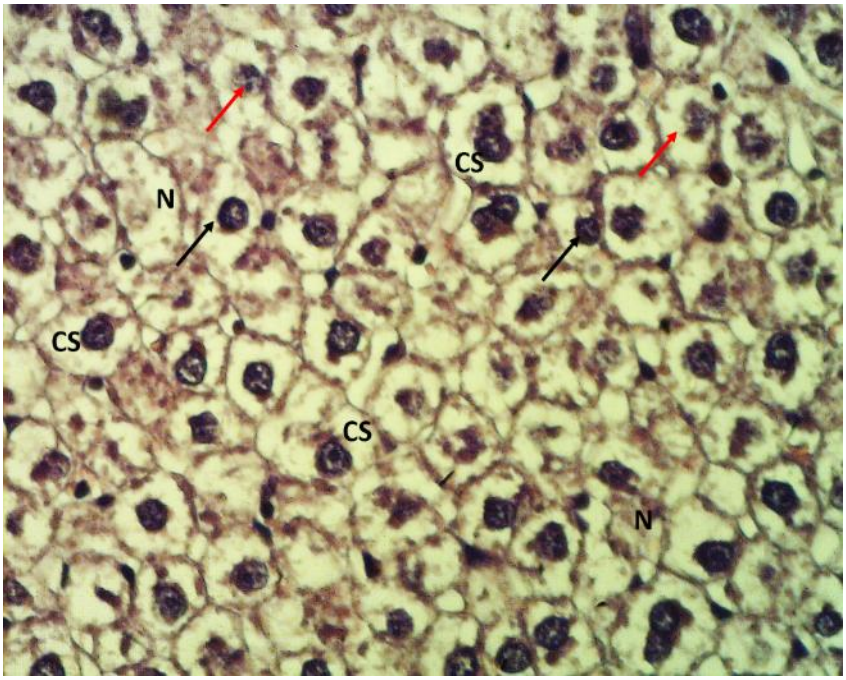

Fig - 3: Magnified section of liver for rats injected with lead acetate a dose of 60 mg/kg shows cellular swelling (CS), necrosis (N), pyknosis (black arrows) and Karyorrhexis (Red arrows). (H&E 400X)

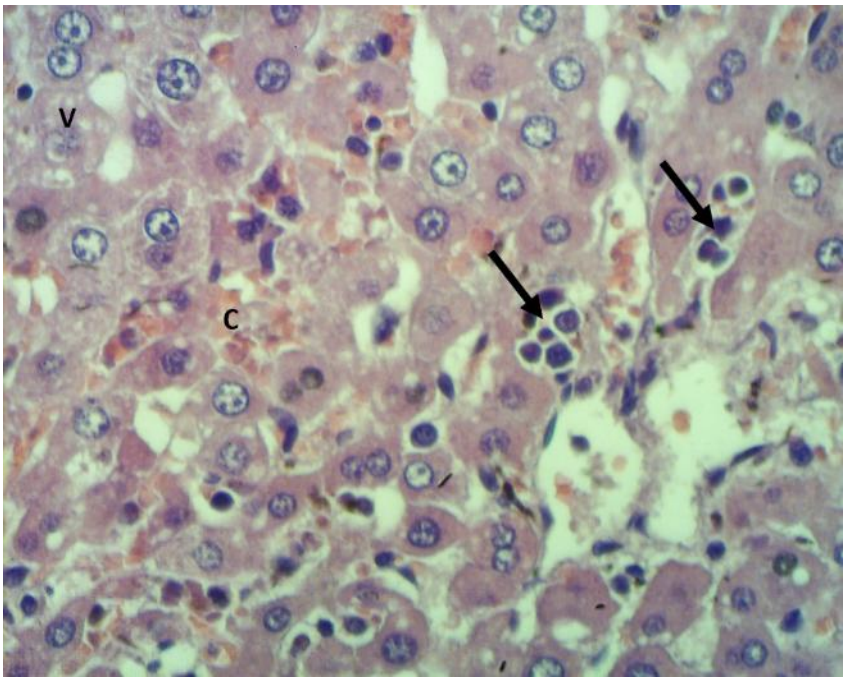

Fig - 4: Magnified section of liver for rats injected with lead acetate a dose of 60 mg/kg + orally 1000 mg/kg *Spirulina* extract shows sinusoidal congestion (C) and hypercellulartiy (arrows) (H&E 400X).

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References

Abu Zeid IEME, 2001. The Effect of Carbofuran and Endosulfan on the African Catfish, Clarias Garieplnus. Ph.D. dissertation, Universiti Putra Malaysia, Malaysia.

Al-Hamdany AS, 2010. The Effect of Lead acetate on histologic structure of liver, kidney, spleen and some blood parameters in the white rats *Rattus rattus*. M.S. thesis, University of Babylon, Iraq.

Al-Yassiry TMH, 2014. Ecological Assessment of the Sewage in The City of Hilla/Iraq Using the Canadian Model and the Study of Phytoplankton. M.S. thesis, University of Babylon, Iraq.

Annabi BA and Nehde A, 2007. Antioxidant enzymes activity and bilirubin level in adult rats treated with lead. Clin. Res. Biol. 330: 581-588.

Ashour AA, Yassin MM, Abu Aasi NM and ALI RM, 2007. Blood, Serum Glucose and Renal Parameters in Lead-Loaded Albino Rats and Treatment with Some Chelating Agents and Natural Oils. Turk. J. Biol. 31: 25-34.

Bancroft JD, 2008. Theory and Practice of Histological Techniques. 6th edition. Churchill living stone. Elsevier Health Sciences. pp: 725.

Belay A, 2002. The Potential Application of Spirulina (Arthrospira) as a Nutritional and Therapeutic Supplement in Health Management. J. Am. Nutraceut. Assoc. 5: 27-48.

Bergmeier W and Stefanini L, 2009. Novel molecules in calcium signaling in platelets. J Thromb Haemost, 7:187-190.

Chu W, Lim Y, Radhakrishnan AK and Lim P, 2010. Protective effect of aqueous extract from *Spirulina* platensis against cell death induced by free radicals. BMC Complement Altern Med. 10:53.

Colla LM, Reinehr CO, Reichert C and Costa JAV, 2007. Production of biomass and nutraceutical compounds by Spirulina platensis under different temperature and nitrogen regimes. Bioresour. Technol. 98: 1489-1493.

Dauqan E, Sani HA, Abdullah A, Muhamad H and Gapor AB, 2011. Vitamin E and Beta Carotene Composition in Four Different Vegetable Oils. Am. J. Appl. Sci. 8: 407-412.

Dong W, Ning L, Lu W and Li C, 2009. Tumor-inhibitory and liver-protective effects of Phellinus igniarius extracellular polysaccharides. World J. Microbiol. Biotechnol. 25: 633–638.

Erlinger TP, Muntner P and Helzlsouer KJ, 2004. WBC count and the risk of cancer mortality in a national sample of US adults: Results from the Second National Health and Nutrition Examination Survey mortality study. Cancer Epidemiol. Biomark. Prev. 13: 1052–1056.

Johnson P and Shubert LE, 1986. Availability of iron to rats from *Spirulina*, a blue-green alga. Nutr. Res. 6: 85-94.

Flora SJS, Saxena G, Gautam P, Kaur P and Gill KD, 2007. Response of lead-induced oxidative stress and alterations in biogenic amines in different rat brain regions to combined administration of DMSA and MiADMSA. Chem. Biol. Interact. 170: 209-220.

Flora SJS, 2002. Nutritional components modify metal absorption, toxic response and chelation therapy. J. Nutr. Environ. Med. 12: 53–67.

Gaoa R, Yuana Z, Zhaob Z and Gaob X, 1998. Mechanism of pyrogallol autoxidation and determination of superoxide dismutase enzyme activity, Bioelectrochem. Bioenerg. 45: 41–45.

Groff JL, Gropper SAS and Hunt SM, 1995. Advanced Nutrition and Human Metabolism, 2nd ed. West Publishing Company, Madison, Wisconsin, USA.

Guidet B and Shah SV, 1989. Enhanced in vivo H_2O_2 generation by rat kidney in glycerol–induced renal failure. Am. J. Physiol. 257: 440-445.

Gupta VK, 2010. Comprehensive Bioactive Natural Products, 1st ed. Stadium Press, LLC, USA.

Gurer H and Ercal N, 2000. Can antioxidants be beneficial in the treatment of lead poisoning? Free Radic. Biol. Med. 29: 927–945.

Hemalatha K, Pugazhendy K, Jayachandran K, Jayanthi C and Meenamba M, 2012. Studies on the Protective Efficacy of *Spirulina* against Lead Acetate Induced Hepatotoxicity in Rattus norvegicus. Int. J. Chem. Anal. Sci. 3: 1509-1512.

Hosseini SM, Khosravi-Darani K and Mozafari MR, 2013. Nutritional and medical applications of *Spirulina* microalgae. Mini. Rev. Med. Chem. 13: 1231-1237.

Huang Z, Guo BJ, Wong RN and Jiang Y, 2007. Characterization and antioxidant activity of selenium-containing phycocyanin isolated from *Spirulina platensis*. Food Chem. 100:1137–1143.

Institute of Medicine, 2001. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. National Academies Press, Washington, USA.

Karadeniz A, Yildirim A, Simsek N, Kalkan Y and Celebi F, 2008. *Spirulina* platensis protects against gentamicin-induced nephrotoxicity in rats. Phytotherap. Res. 22: 1506-1510.

Kumar KS, Ganesan K and Subba Rao PVS, 2008. Antioxidant potential of solvent extracts of Kappaphycus alvareii (Doty) Doty –An edible seaweed. Food Chem. 107: 289-295.

Linden AH, Gülden M, Martin H and Seibert H, 2008. Peroxide induced cell death and lipid peroxidation in glioma cells. Toxicol. Vitro. 22: 1371-1375.

Mahakhart A, Sano T, Ratanachot P, Tong-a-ram T, Srivastava VC, Watanabe MM and Kaya K, 1998. Detection of microcystins from cyanobacterial water blooms in Thailand fresh water. Phycological Res. 46: 25-29.

Marreiro DD, Cruz KJ, Morais JB, Severo JS and Oliveira AR, 2017. Zinc and Oxidative Stress: Current Mechanisms. Antioxidants. 6: 1-9.

Maulood BK, Hassan FM, Al-Lami AA, Toma JJ and Ismail AM, 2013. Checklist of Algal Flora in Iraq. Published in Ministry of Environment, Baghdad-Iraq.

Patrick L, 2006. Lead toxicity part II: the role of free radical damage and the use of antioxidants in the pathology and treatment of lead toxicity. Altern. Med. Rev. 11: 114–127.

Pierlovisi C, 2007. L'Hommeetla Spiruline: Un avenir commun? Composition chimique, intérêts alimentaires et activités biologiques. Ph.D. dissertation,Paris Descartes University, Paris.

Ravi M, Lata S, Azharuddin S and Paul SF, 2010. The Beneficial Effects of *Spirulina* Focusing on its Immunomodulatory and Antioxidant Properties. Nutr. Diet. Suppl. 2: 73-83.

Ross CA, Taylor CL, Yaktine AL, and Valle HBD, 2011. Dietary Reference Intakes for Calcium and Vitamin D: Institute of Medicine, National Academies Press, Washington (DC), USA.

Sahu RK, Kar M and Routray R, 2013. DPPH free radical scavenging activity of some leafy vegetables used by tribals of Odisha. Indian J. Med. Plants Stud. 1: 21-27.

Samuels R, Mani UV, Lyer UM and Nayak US, 2002. Hypocholesterolemic effect of *Spirulina* in patients with hyperlipidemic nephrotic syndrome. J. Med. Food. 5:91-96.

SAS (Statistical Analysis System), 2012. Statistical Analysis System, User's Guide Statistical, 1st ed. SAS. Inst. Inc. USA.

Sawidis T, Brown MT, Zachariadis G and Sratis I, 2001. Trace metal concentrations in marine macroalgae from different biotopes in the Aegean Sea. Environ. Inter. 27: 43-47.

Schafer FQ, Wang HP, Kelley EE, Cueno KL, Martin SM and Buettner GR, 2002. Comparing beta-carotene, vitamin E and nitric oxide as membrane antioxidants. Biol. Chem. 383: 671-681.

Teneva I, Dzhambazov B, Koleva L, Mladenov R and Schirmer K, 2005. Toxic potential of five freshwater Phormidium species (Cyanoprokaryota). Toxicon. 45: 711-725.

Urmila J, Anish JZ, Kumar P, Bisen PS and Prasad JB, 2012. Alleviation of metabolic abnormalities induced by excessive fructose administration in Wistar rats by *Spirulina* maxima. Indian J. Med. Res. 135:422–428.

Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M and Telser J, 2007. Free radicals and antioxidants in normal physiological functions and human disease. Rev. Int. J. Biochem. Cell Biol. 39: 44–84.