التأثيرات المناعية للطحالب الدقيقة *Chlorella spp* على الفئران المصابة بالتسمم الكبدى.

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الملخص العربى

تندرج الطحالب الخضراء (*Chlorella spp*) ضمن مملكة الطلائعيات، وهي مملكة منفصلة تمامًا عن كل من المملكتين النباتية والحيوانية، إلا أنّ لها بعض الخصائص المشتركة مع كليهما مثل إنتاج الطاقة من خلال عملية البناء الضوئي مثلًا، وأهم ما يميّز هذا النوع من الكائنات الحيّة هو أنّ بعضها أحادية الخلايا، وحقيقيّة النواه و تتميز الطحالب الخضراء بوجود الأصباغ الضرورية للقيام بعملية البناء الضوئي؛ كالكلوروفيل، والزاثوفيل، وتتواجد هذه الأصباغ في البلاستيدات تبعًا لنوع الطحالب. تهدف هذه الدراسة إلى التعرف على التأثيرات المناعية للطحالب الدقيقة *Chlorella spp* على الفئران المصابة بالتسمم الكبدي . استخدمت الدراسة ٣٠ فأر البينو ابيض يوزن كل منها ١٧٠ جرامًا وعمرها عشرة أسابيع ، وتم تقسيم الفئران الى خمس مجاميع وكل مجموعة تحتوى على ٦ فئران و نجد ان اربع مجاميع تم حقنهم برابع كلوريد الكربون لأحداث التسمم الكبدي و منهم المجموعة الضابطة الموجبة و هي مجموعة فئران مصابة بالتسمم الكبدي و تتغذى على الوجبة الأساسية فقط و أيضا الثلاثة المجاميع المتبقية تم تغذيتهم على الغذاء التجريبي (الوجبة الأساسية بالإضافة الى ثلاثة جرعات من الطحلب (٥, ١٠ , ١٥%) لمدة شهر. وفى نهاية التجربة تم ذبح الفئران وتم جمع عينات الدم لإجراء التحاليل البيوكيميائية أظهرت النتائج ان المجموعة الثالثة (٥%) تحسن في أعراض MAD عند مقارنتها بالمجموعة الضابطة السالبة ويمكن التوصية بتناول بجرعات متنوعة من الطحالب لتحسين الاستعداد المناعي لمرضى الكبد.

الكلمات المفتاحية : Chlorella spp **– التغيرات المناعية – التسمم الكبدى**

Immunological effects of microalgae *Chlorella spp* on hepatotoxic rats.

Abstract:

The single-celled green algae of the Chlorophyta division, of which there are around thirteen distinct species in the genus Chlorella, are all chlorophytes. The cells are spherical, with diameters between two and ten m, and they lack flagella. Chlorophylls-a and -b, green photosynthetic pigments, are found in their chloroplasts. This exploratory study intends to learn about the immunological effects of the microalgae Chlorella spp on hepatotoxic rats. Chlorella cells proliferate quickly in favorable settings, requiring just carbon dioxide, water, sunshine, and a trace quantity of minerals. For seven days prior to the experiment, the rats were kept in a cage (animal shelter). Two groups of rats were created; the first (n=6) received only food. Those who followed a baseline diet for 28 days served as the diet's opposite, positive

control group (C-ve). The second set of rats were exposed to Carbon Tetrachloride (n= 24 rats), and they were given 5persent, 10persent, and 15persent Chlorella spp whereas the control rats received a positive control. Group 3 demonstrated the greatest improvement in MAD symptoms compared to the other groups, and when contrasted with the control group of healthy rats, this group's therapy clearly outperformed the others. There were no discernible changes between the third and fourth group-fed rats. Group 4 (10percent Chlorella spp.), as compared to the control group, had numerically superior serum (sod.). When comparing the (WBC) of rats fed on treatments 3, 4, and 5, there were no statistically significant changes compared to the control (+) group. Chlorella spp., a kind of microalgae, may be prescribed in a variety of dosages to improve the immunological preparedness of hepatic patients.

Keywords: Chlorella spp **,** Immunological effects hepatoprotective, intoxicated Rats.

1. INTRODUCTION

The single-celled green algae of the Chlorophyta division, of which there are around thirteen distinct species in the genus Chlorella, are all chlorophytes. Cells have a spherical form, range in size from around two to ten μm, and lack flagella. Chlorophylls-a and -b, green photosynthetic pigments, are found in their chloroplasts. With just carbon dioxide, water, sunshine, and a trace mineral supplement, Chlorella cells may quickly divide and replicate under favorable circumstances (**Schulz *et al*., 2022).** Certain "Chlorella" food strains have been misidentified, or their taxonomic placements do not match those of authentic Chlorella. Chlorella luteoviridis, the common name for Heterochlorella luteoviridis, is no longer accepted as a valid name. **(Champenois *et al*., 2015),** Animal research has shown some interest for chlorella's potential to eliminate pesticides from the body. Detoxification of chlordecone, a persistent pesticide, was sped up by the presence of Cholera protothecoides in chlordecone-poisoned rats, shortening the toxic substance's half-life from forty to 19 days. The algae were digested without incident by the digestive system, which then eliminated the bound chlordecone with the feces by preventing the enteric recirculation of the persistent pesticide **(William, 2020).** The benefits of chlorella as a food source are numerous. Around 50–60percent of it is protein, and of that, about a third is complete protein, meaning it contains all nine essential amino acids. So, it is a perfect choice for vegans and vegetarians as well as anybody looking to increase their protein intake**. (Khanra *et al*.,2018)** . In addition, research shows that the minerals in chlorella aid in reducing bad (LDL) cholesterol and triglycerides, both of which can lead to artery blockage and cardiac stress. The

chlorella's fatty acids and minerals, such potassium, also relax blood vessels. The health of the circulatory system is enhanced and blood pressure is reduced as a result. Preventing heart disease requires keeping vitals like cholesterol, blood pressure, and blood arteries in excellent shape**. (Muys *et al.*,2018).** Studies have shown that the antioxidant violaxanthin, present in leafy greens like chlorella, can help decrease inflammation. Further investigation has revealed that other antioxidants in chlorella, such as lycopene, also play a role in this phenomenon. Chronic inflammation is linked to diabetes, heart disease, gastrointestinal diseases, and arthritis, per the World Health Organization (WHO). The leading global killer is inflammation. Omega-3s fatty acids, vitamin C, and carotenoids like beta-carotene and lutein are just a few of the many antioxidants found in chlorella. These nutrients protect cells from becoming damaged, which in turn lowers your chance of developing diabetes, dementia, heart disease, and cancer. (**Bleakley *et al.*,2017).** Chlorella's antioxidants and other nutrients have been studied for their potential to fight cancer, viruses, and germs. Clinical trials showed that chlorella raised white blood cell numbers, which can boost the immune system and aid in the battle against illness. **(Enzing *et al.*, 2014)**

Being immune means that your body can detect and get rid of harmful invaders. The immune system is the body's natural defense mechanism against foreign organisms and particles that have penetrated the skin or mucous membranes. Yet, the stress-induced hormonal and metabolic changes suppress the immune system's ability to fight against disease. Impaired immune system is already weakened by stress, but adding hunger to the mix just makes things worse. The danger of illness increases when immunity is weakened, nutrition reduces when sick, and immunity declines when the body isn't properly nourished. (**AN *et al.*,** ٢٠١٦). A common pathology, chronic liver damage includes the stages of steatosis, chronic hepatitis, fibrosis, cirrhosis, and hepatocellular cancer. Antioxidants have been proposed as therapeutic agents and pharmacological coadjuvants to prevent and treat liver damage caused by oxidative stress, which plays a pivotal role in the development and progression of liver disorders. In this work **(AN *et al.*, 2000)**

2. THE PURPOSE OF THE STUDY:

3. Understanding the immunological effects of the microalgae Chlorella spp. in hepatotoxic rats was the focus of this research.

4. MATERIALS

4.1. Freshwater Algae

4.2. The chlorella spp. freshwater algae were purchased dried from a neighborhood store and isolated.

4.3. Diets

4.3.1 The Basic Diet

- For example, 10percent of the Basel diet was casein, 10percent was maize oil, 1percent was a vitamin and mineral blend, 4percent was a salt and seasoning blend, 0.2percent was choline chloride, 0.3percent was methionine, 5percent was cellulose, and 69.5percent was corn starch. Source **Reeves et al., (1993).**
- The basal diet in the test contained CaCO3 (600 mg), Ca HPO4. 2H2O (150 mg), MgSO4.2H2O (204 mg), Nacl (334 mg), Fe (C6H5O7) 26H2O (55 mg), Kl (1.6 mg), MnSO4.4H2O (10 mg), K2 HPO4 (645 mg), (150 mg), MgSO4.2H2O (204 mg), Zncl2 (0.5 mg) and Cu SO4. 5H2O (0.06 mg) Source **(Hegsted *et al.*, 1941).**
- The test's baseline diet included Vitamin E (10 Iu), Thiamin (0.50 mg), Vitamin K (0.50 Iu), Vitamin A (200 Iu), , Pyridoxine (1.00 mg), Niacin (4.00 mg), Calcium panthothenic acid (0.40 mg), Folic acid (0.02 mg) ,Vitamin D (100 Iu), Choline chloride (200 mg), Inositol (24 mg), Para-amino – benzoic acid (0.02 mg), **(Campbell, 1963)**
- **Experimental diet**

Table (1): The composition of basal and Experimental diet:

Component (g)	Basal diet	Basal diet +5% Freshwater algae	Basal diet +10% Freshwater algae	Basal diet +15% Freshwater algae
Test ingredients	-	5	10	15
Casein	20	20	20	20
Corn oil	4.7	4.7	4.7	4.7
Mineral mix	3.5	3.5	3.5	3.5
Vitamin mix	1	1	1	1
Cellulose	5	5	5	5
Choline chloride	2	2	2	2
Sucrose	10	10	10	10
Corn starch	Up to 100	Up to 100	Up to 100	Up to 100

4.4. Carbon tetrachloride (Ccl4)

Carbon tetrachloride (Ccl4) was sent from El-Gomhoria Company for Chemical Industries to Cairo, Egypt. They used a 10% liquid solution to give it to the patients. According to (Passmore & Eastwood, 1986), it was distributed in one-liter white plastic water bottles containing a harmful chemical component for liver illness. It's diluted with drugstore-bought paraffin oil before being inhaled during induction **(Passmore & Eastwood, 1986).**

4.5. Rats

Thirty (30) mature male Sprague-Dawley rats weighing 150-160 g B.Wt. at 14-16 weeks were utilized. Unsanitary plastic cages with metal roofs were used to house the animals. Before the experiment, rats were fed the standard food for seven days to facilitate adaptation. Water was accessible on demand from a bottle with a narrow opening that was connected to a metal tube and plastic tubing. The rats, as was noted before, were habituated to a 12-hour light/12-hour dark cycle for seven days prior to the commencement of the study.

4. METHODS

4.6. Preparation of plant

Dried freshwater algae were purchased from a vendor at an open air market in al Baha, Saudi Arabia. All the plant materials were ground up in a blender and kept in a dark-topped glass jar in the fridge until needed. **Russo (2001)** recommends storing all herbs and plants in a cold, dark, and dry place to prevent oxidation. Carbon tetrachloride (Ccl4) in 50percent V/V paraffin oil (2ml / kg b. wt.) was subcutaneously injected into 20 male albino rats twice weekly for two weeks to cause chronic liver damage, as reported by **Jayasekhar et al.(1997).** Retro-orbital blood was drawn from subjects after Ccl4 injection to assess liver health and function.

Rats are grouped and fed.

Thirty 150-l60-gram Sprague Dawley white male albino rats were employed in the experiment. There were six rodents in each of the 5 groups. Below are the several types of rats:

- **Group (1):** In the untreated group, rats were given a regular meal (control"-").
- **Group (2):** Rats on a regular diet who were also given carbon tetrachloride made up the control positive group (control "+"). (CCl4).
- **Group (3)** was fed a regular diet with the addition of five percent Freshwater algae.
- **Group (4)** got a regular diet with 10percentage added Freshwater algae.
- **Group (5)** fed 15percentage Freshwater algae in addition to the regular diet.

Blood sampling

- To conclude the experiment, the rats were put to death while under the effects of ether (28 days). Using the retro-orbital approach, blood samples were collected in a sterile centrifuge tube. They were allowed to get to a coagulated state at room temperature before being centrifuged at 1500 rpm for 15 minutes. Following collection of serum with a wash and dry syringe, the samples were transferred to Wasserman tubes and stored at -10 °C for biochemical analysis. The livers, spleens, lungs, hearts, and kidneys of rats were removed, washed in saline, weighed, and dried. We used the method described below to determine the weights of the organs that were listed. Organs were formalin-fixed (10% V/V), as reported by **Drury & Wallington (1967),** prior to histological examination.
- **Biological evaluation:**

  It has been shown by Chapman D.G. (1959) that there is a correlation between FER, food intake (consumption), bodyweight gain percentage (BWG percent), and FER. The following formula will be utilized:

$$BWG\% = \frac{Final\ weight - Initial\ weight}{Initial\ weight} \times 100$$

$$FER = \frac{Gain\ in\ body\ weight\ (g\ /\ day)}{Food\ Intake (g\ /\ day)}$$

$$\text{The organs' relative weight} = \frac{\text{Organs weight}}{\text{Animal body weight}} \times 100$$

4- Biochemical analysis

- The level of glutathione was determined using the procedure provided by **(Elinan, 1959). (Raja et al., 2007).** Enzyme levels of glutathione peroxidase, glutathione reduced, and superoxide dismutase in the serum were measured using a commercial kit and an autoanalyzer **(Roche-Hitachi, Japan) (Hissin and Hiff ,1976); (Kakkar *et al.*, 1984) and (Sinha ,1972),** respectively.
- The Amount of Malondialdehyde (MDA) Present Concentrations of malondialdehyde were measured colorimetrically using a thiobarbituric acid reactive substance (TBARS) test kit (**Wasowich *et al.*, 1993).**

5. Analytical statistics

SPSS was used for the automatic statistical analysis (Statistic Program statistical software, SAS Institute, Sigmastat, Cary, NC). To determine if there was a statistically significant difference between the groups, one-way analysis of variance (ANOVA) and Duncan's multiple tests were used to examine the treatment effects (p less than 0.05) **(Snedecor and Cochran, 1967).**

6. RESULTS AND DISCUSSION

Effect of different level of Microalgae *Chlorella spp* on serum (GSH) (|imol\L)of Hepatointoxicated rats.

Serum glutathione (GSH) (imol/L) levels of rat hepatocytes exposed to various diets are shown in table (2). The average (GSH) levels in the control (+) group were 5.44±1.87 and 8.98±1.33 in the control (-) group, respectively, indicating a significant difference with a percent decrease of -39.42percent of control (±) group as compared to control (-) group. In contrast to the control (+) group, all dietary treatments for hepatic rats resulted in statistically significant increases in mean values. (6.43± 1.45 imolL, 6.59 ± 2.01imol\L, and 7.22 ± 1.98) 5 persent, Ten persent, and 15 percent for Chlorella species. Groups 3, 4, and 5 saw increases of 18.20%, 21.14%, and 32.72%, respectively. Nonsignificant changes were found in the rats fed from groups 3 and 4. Similar variations were seen in rats fed all diets. In contrast to the "+"" control group. Group 5 (15percent Chlorella spp.), when compared numerically to the control group (-), revealed significantly higher levels of blood glutathione (GSH). These numbers line up with what was previously published by **(Salman et al., 2008).** Glutathione is the most abundant non-protein thiol in living creatures, and it plays a crucial role in

regulating the body's intrinsic antioxidant defense mechanisms. **(Gueeri, 1995).** According to studies, reduced glutathione (GSH) is crucial for eliminating reactive harmful compounds **(Recknagel et al., 1991).**

Table (2): The effect of feeding different amounts of Microalgae *Chlorella spp* **on antioxidants (serum glutathione, GSH, (imol/L) levels in Hepatointoxicated rats.**

Value	Control (-)	Control (+)	Microalgae *Chlorella spp*		
			5%	10%	15%
Range	7.20-10.11	3.02-8.17	5.03-8.52	4.10-8.45	4.89-9.65
Mean	8.98^{a}	5.44^{d}	6.43^{c}	6.59^{c}	7.22^{b}
SD	1.33	1.87	1.45	2.01	1.98
%of Change	0.00	-39.42	18.20	21.14	32.72

Values in the same row with different superscript litters are significantly different at P<0.05. (Gl) conrtol- (G2)control+ (G3) *Chlorella spp* 5% (G4) *Chlorella spp* 10%(G5) *Chlorella spp* **15%**

Effect of different level of Microalgae *Chlorella spp* on serum antioxidants (serum Glutathione peroxidase, GSH-Px, U/g Hb) of Hepatointoxicated rats.

Table 3 shows the serum glutathione peroxidase (GSH-px) (U/g Hb) of hepatic rats on four different diets. By comparing the control (+) and control (-) groups, it was clear that the control (+) group had a lower mean value of (GSH-px) (12.79±3.11 vs. 24.56±2.98), representing a statistically significant difference (-47.92%). In contrast to the control (+) group, all dietary treatments for hepatic rats resulted in statistically significant increases in mean values. The values were 16.66±2.01, 17.45 ±3.17 and 18.54± 1.86 U/g Hb. for **(***Chlorella spp 5%,10% and 15%)* respectively. For groups 3, 4, and 5, the respective growth rates were 30.26, 36.43, and 44.96%. Feeding experiments on rats with all of groups 3, 4, and 5 showed no statistically significant differences. Similar variations were seen in rats fed all diets. In contrast to the "plus" control group. Group five (Chlorella spp 15%) had statistically higher serum (GSH-px) levels compared to the control (-) group. Consistent with the findings of **(Aly et al., 2017),** the current study found that NDEA (Nitrosoethanamine, Diethylnitrosamine) induced hepatocellular injury characterized by a substantial decrease in heptic GSH, GPx, and CAT activitis, which was subsequently ameliorated by the administration of antioxidants. It has been shown that supplementation with cactus-pear fruit reduces plasma levels of 8-epi-

prostaglandin F2a (PGF2a) and oxidative stress-related molecules, MDA, by roughly 30percent and 75persent, respectively. Red blood cell measurements of GSH and its oxidized counterpart, GSSG, showed an increase, indicating less oxidative damage and a greater reducing capability. After a washout period of 6 weeks, the same people were given a vitamin C supplement at the same frequency and in the same dose as when they ate the fruit. This supplement was taken for 15 days. No oxidative stress markers were influenced by vitamin C supplementation. To sum up, it was determined that healthy humans who ate cactus-pear fruits had better redox balance, less oxidative damage to lipids, and higher antioxidant status than those who didn't **(Salman *et al,* 2008).**

Table(3): The effect of feeding different amounts of Microalgae *Chlorella spp* on biological antioxidants (serum Glutathione peroxidase, GSH-Px, U/g Hb) levels in Hepatointoxicated rats.

Value	Control (-)	Control (+)	Microalgae *Chlorella spp*		
			5%	10%	15%
Range	20.78-31.56	10.56-15.67	15.01-18.43	14.67-19.54	14.67-21.86
Mean	24.56^{a}	12.79^{c}	16.66^{b}	17.45^{b}	18.54^{b}
SD	2.98	3.11	2.01	3.17	1.86
%of Change	0.00	-47.92	30.26	36.43	44.96

Values in the same row with different superscript litters are significantly different at P less than 0.05. (GSH-Px, U/g Hb)

Effect of different level of Microalgae *Chlorella spp* on serum (CAT) (U/g Hb) of **Hepatointoxicated rats.**

The serum catalase activity (CAT) (U/g Hb) of hepatic rats given the various diets is shown in table (4). Comparing the control (+) and control (-) groups, it was clear that the control (+) group had a lower mean value of (CAT), with 118.56±20.67 and 184.20±24.67, respectively, indicating a significant difference with a decrease of -35.64 percent for the control (+) group. In contrast to the control (+) group, all dietary treatments for hepatic rats resulted in statistically significant increases in mean values. The values were 159.45±1 1.56, 161.67 ±16.76 and 168.78±8.55 U/g Hb. for (*Chlorella spp* 5%, 10% and 15%) respectively. Groups 3, 4, and 5 saw increases of 34.49 percent, 36.36 percent, and 42.36 percent, respectively. Nonsignificant differences were found between the groups after all rats were fed from groups 3, 4. Similar variations were seen in rats fed all diets. In contrast to the "+"" control group. Statistically, group five (Chlorella spp 15%) had a higher CAT than the control group (-). Current findings are consistent with those reported by **(Ben-Saad et al., 2017).** Shown hepatoprotective activity in a rat model of lithium-induced liver damage.

Lithium-induced alterations in the liver's histopathology (such as sinusoidal dilatation, clogged central veins, vacuolization, and infiltrating inflammatory cells) were mitigated in rats fed cladodes extract. Once cladode extract was injected into the liver, CAT, SOD, and GPx activities were significantly increased.

Table (4): **Effect of different level of Microalgae *Chlorella spp* on biological antioxidants (serum Catalase (CAT, U/g Hb) levels of Hepatointoxicated rats.**

Value	Control (-)	Control (+)	Microalgae *Chlorella spp*		
			5%	10%	15%
Range	146.45-190.55	106.78-132.76	135.24-180.56	142.67-184.67	154.67-180.23
Mean	184.20^{3}	118.56^{d}	$159.45b^{c}$	$161.67b^{c}$	168.78^{b}
SD	24.67	20.67	11.56	16.76	8.55
%of Change	0.00	-35.64	34.49	36.36	42.36

Values in the same row with different superscript litters are significantly different at P less than 0.05. (CAT, U/g Hb).

Effect of different level of Microalgae *Chlorella spp* on serum (SOD) (U/g Hb) of **Hepatointoxicated rats.**

Hepatic rats were fed the diets listed in table (5), and their serum superoxide dismutase (SOD) (U/g Hb) levels were compared. The control (+) group had a lower mean value of (SOD) (3.23±0.59) compared to the control (-) group (5.45±1.11). This difference was statistically significant. That's a decrease of (-40.73 percent drop). In contrast to the control (+) group, all dietary treatments for hepatic rats resulted in statistically significant increases in mean values. The values were 4.17±0.89, 4.20 ±1.54 and 4.38±0.99 U/g Hb. for (*Chlorella spp* 5%, 10% and 15%)respectively. Groups 3, 4, and 5 saw increases of 29.10%, 30.033%, and 35.60%, respectively. There were no discernible differences between groups three, four, and five in the rat diet. Similar variations were seen in rats fed all diets. In contrast to the "+"" control group. In a numerical comparison, group five (Chlorella spp15%) had significantly higher serum superoxide dismutase levels than the control group (-). According to **(Salman et al., 2008),** our current findings are consistent with theirs. Glutathione, a thiol that is not a protein, is essential for the regulation of the body's natural antioxidant defenses **(Gueeri, 1995).** According to studies, reduced glutathione (GSH) is crucial in the elimination of reactive hazardous metabolites **(Recknagel et al., 1991).**

Table (5): Effect of different level of Microalgae *Chlorella spp* on biological antioxidants (serum Superoxide dismutase, SOD, U/gHb) levels of Hepatointoxicated rats.

Value	Control (-)	Control (+)	Microalgae *Chlorella spp*		
			5%	10%	15%
Range	3.78-7.54	2.99-4.54	3.15-5.78	2.89-5.41	3.11-6.01
Mean	5.45^{a}	3.23°	4.17^{b}	4.20^{b}	4.38^{b}
SD	1.11	0.59	0.89	1.54	0.99
%of Change	0.00	-40.73	29.10	30.03	35.60

Values in the same row with different superscript litters are significantly different at P<0.05.(SOD, U/g Hb).

Effect of different level of Microalgae *Chlorella spp* on biological oxidants (serum malonaldehyde concentration, MDA, nmol /mL) of Hepatointoxicated rats.

The serum malondialdehyde (MDA) levels (nmol/mL) of rats with diet-induced hepatic steatosis are shown in table (6). Compared to the control (-) group, the (+) group had a much higher mean value of (MDA), at 4.13±0.78 versus 2.76±0.45; this represented a 49.64% increase in the control (+) group. In contrast to the (+) group, all dietary treatments for hepatic rats resulted in statistically significant increases in mean values. The values were 3.33±0.67, 3.30 ±1.01 and 3.04±0.55 nmol / mL. for (*Chlorella spp* 5%,10%,15%) respectively. The % of decreases were -19.38 -20.08 and -26.39 % for groups three, four, and five respectively. There were no discernible differences between groups 3, 4, and 5 in the rat diet. Similar variations were seen in rats fed all diets. As compared to control (±) group. Group 5 (Chlorella spp 15%) had significantly lower levels of serum malondialdehyde oxidase (MDA) equated to the control group (-). are consistent with what has been reported by **(Salman et al., 2008).** Glutathione, a thiol that is not a protein, is essential for the regulation of the body's natural antioxidant defenses **(Gueeri, 1995).** Reduced glutathione (GSH) plays a key role in the detoxification of the reactive toxic metabolites as reported by **(Recknagel et al., 1991).**

Table (6): Effect of different level of Microalgae *Chlorella spp* on biological oxidants (serum malonaldehyde concentration, MDA, nmol /mL)of Hepatointoxicated rats.

Value	Control (-)	Control (+)	Microalgae *Chlorella spp*		
			5%	10%	15%
Range	1.99-3.56	3.56-5.12	2.87-3.98	2.65-4.11	2.67 -4.01
Mean	2.76^{c}	4.13^{a}	3.33^{b}	3.30^{b}	3.04^{b}
SD	0.45	0.78	0.67	1.01	0.55
%of Change	0.00	49.64	-19.38	-20.08	-26.39

In the same table, values with different superscripts are statistically different at the P less than 0.05 level. (MDA, nmol / mL)

Effect of different level of Microalgae *Chlorella spp* on Weight blood cells (WBCS) (mml of Hepatointoxicated rats.

Total white blood cell counts in the serum of hepatically-isolated rats given a variety of diets are shown in table (7). One can see that the mean value of white blood cell count (WBC) in the control (-) group is greater than in the control (+) group (21.66 ± 3.71 vs. 19.93 ± 4.55). There were statistically significant increases in the mean values of all hepatic animals fed experimental meals compared to the control (+) group. The values were 18.73 ±.66, 315.43 ± 4.47and 17.40 ±4.09nmol / mL. for (*Chlorella spp* 5%,10%,15%) respectively. There were no discernible differences between groups 3, 4, and 5 in the rat diet. Similar variations were seen in rats fed all dietsCompared to the placebo group (-). Chlorella's antioxidants and other nutrients have been studied for their potential to fight cancer, viruses, and germs. Clinical trials showed that chlorella raised white blood cell numbers, which can boost the immune system and aid in the battle against illness **(Enzing *et al.*, 2014)**

Table (6): The Impact of Chlorella spp. Microalgae Amount on Serum Protein Content in Rats (total white blood cells) of Hepatointoxicated rats.

Parameters Groups	WBC
	Mean ± SD
Control (-)	21.66 ±3.78b
Control (+)	19.93 ±4.55a
Chlorella spp **5%**	18.73 ±.66a
Chlorella spp **10%**	15.43 ± 4.47a
Chlorella spp **15%**	17.40 ±4.09a
F Value	.872
P Value	.577

Data represented as (Mean + SD) significance (P less than 0.05) between control positive and other treatment.

CONCLUSIONS AND RECOMMENDATIONS

A higher rate of improvement was seen in the 5% (microalgae Chlorella spp) group, demonstrating that the microalgae Chlorella spp has a beneficial impact in enhancing immunological preparties in hepatic rats. This is because of a synergistic action in enhancing antioxidant defense capabilities, which in turn protects liver tissues from toxicity. These extracts can be given to patients as part of a larger herbal treatment. It might be suggested that:

- Chlorella spp. microalgae are recommended for hepatic patients.
- The varying sum of Microalgae *Chlorella spp* , For hepatic patients, in particular, that of 5persent, is helpful.
- • Many dosages of Microalgae *Chlorella spp* may be recommended for enhancing the immunological preparties for hepatic patients.

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