Hepatotoprotective Activity of Different Doses of *Spirulina* against Ccl4 Induced Liver Damage in Rats

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Abstract: Spirulina is a type of microscopic blue-green algae that is rich in protein, vitamins, minerals, and carotenoids, antioxidants that can help protect cells from damage. The present study was performed to evaluate the effect of different doses of Spirulina on lipids profile and liver and kidney functions in cirrhotic rats by carbon tetrachloride (Ccl_4). Rats were divided into five groups; control groups (1&2) negative and positive were fed on basal diet without supplementation. All treated cirrhotic groups (3-5) were fed on experimental diets with Spirulina by different levels (0.25, 0.5 &1%). Results clearly revealed that the best treatment were Spirulina (1%) which had lowest values of total lipid , triglycerides , total cholesterol, LDL, VLDL, AST, ALT and had the highest values of HDL. While, all groups fed on basal diet with spirulina by different levels (0.25, 0.5 &1%) showed significantly decrease of serum total lipid, triglycerides, total cholesterol LDL, VLDL, AST, ALT and had significant increase of serum HDL, respectively. It could be concluded that Spirulina by different levels (0.25, 0.5 &1%) improve lipids profile and liver functions especially Spirulina by (1%) which has a best significant protective effect against acute hepatotoxicity induced by carbon tetrachloride (Ccl_4) in rats.

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Key Words: Spirulina – cirrhosis – CCl4 – liver oxidative stress.

1. Introduction

During last few years, attention on the microalgae as sources of novel was increased especially biologically active compounds such as phycobiline, phenols, terpenoids, steroids and polysaccharide **(Qi et al., 2006), (Li et al 2007) and (Abd El-Baky et al., 2008).** However, the occurrence of phenolic compounds in blue green algae is less documented than that in higher plants **(Santoyo et al., 2006 and Colla et al 2007).** Algal phenolic compounds were reported to be a potential candidate to combat free radicals, which are harmful to our body and food systems **(Adamson et al., 1999 and Estrada et al., 2001)**.

It is well established that CCl4 induces hepato-toxicity by e cytochrome P450 mediated reactions to produce CCl4–derived radicals such as trichloromethyl (•CCl3) and trichloromethyl peroxyl (•OOCCl3) **(Santoyo et al., 2006)**. These activated radicals bind covalently to the macromolecules (lipids and proteins) and induce peroxidative degradation of membrane lipids that rich in polyunsaturated fatty acids and finally to cell death **(Johnston and Kroening, 1998).** Moreover, this oxidative process is one of the principal causes of hepatotoxicity of CCl4 **(Cotran et al., 1994 and Opoku et al., 2007).**Therefore, the antioxidant activity and/or the inhibition of free radical generation are important in terms of protecting the liver from CCl4– induce damage **(Opoku et al., 2007).**

According to **Cervato et al., (2000) and Athukorala et al., (2006)** who demonstrated that phenolics metabolites are effective in the inhibition of all phases of the peroxidative process: first neutralizing free radicals, then blocking the peroxidation catalysis by oxidizing agent and finally through interruption of lipid–radical chain reactions.

Spirulina, filamentous and unicellular algae is a cyanobacterium belonging to the Oscillatoraceae family that usually grows in the alkaline waters of Africa, Asia, North and South America **(Ciferri, 1983).** Spirulina has been used as food additive because of its high content of proteins as well as essential nutrients like carotenoids, vitamins and minerals **(Belay et al., 1996), (Chamorro et al., 1996) and (Khan et al., 2005 [a]).** In addition, previous studies have demonstrated its several biological activities such as inhibit viral replication **(Hayashi et al., 1996), (Ayehunie et al., 1998) and (Khan et al., 2005 [b]),** prevent anemia **(Kapoor and Metha 1998)**, decrease genotoxicity induced by drugs **(Premkumar et al., 2004)**, prevent fatty liver disease **(Torres et al., 1998), (Vadiraja et al., 1998) and (Torres et al., 2006)** and has hypoglycemic **(Parikh et al., 2001)** and hypolipidemic properties **(Mazo et al., 2004 and Ble-Castillo et al., 2005)**. It has also been studied its effects on vasomotor responses on aortic rings proposing its antihypertensive activities in experimental models **(Mascher et al., 2006 and Paredes et al., 1997)**. In vitro studies demonstrated that the Spirulina and Nestoc species have several therapeutic properties, due to their ability to scavenge superoxide and hydroxyl radicals and inhibit lipid peroxidation **(Li et al., 2007) and (Khan et al., 2005)**. The influence of growth conditions on the chemical composition of Spirulina has been studied by many

researchers with the purpose of optimizing the production of economically and nutritionally interesting compounds, especially pigments, antioxidants vitamins and phycocyanin **(Tanticharoen et al., 1994) and (Abd El-Baky et al., 2009).** However, the manipulating growth conditions for biomass production and productivity are usually used in the commercial production of potentially useful compounds such as ω-3 fatty acids, carotenoids and phenolics **(Abd El-Baky et al., 2007)**.This study aimed to evaluate the effect of different doses of *Spirulina* on lipids profile and liver and kidney functions in cirrhotic rats by carbon tetrachloride (CCl_4).

Materials and Methods:

Materials:

Spirulina (algae):

Freeze dried powder of spirulina (*Spirulina plateniss*) which was obtained from Dainippon Ink and Chemicals, Inc. (Tokyo, Japan), was kindly donated by Biotech Co. (Cheonan, Korea).

Chemicals:

Tetra-chloro-carbon Ccl4 was obtained from El-Gomhorya Company for Chemical and Pharmaceutical, Cairo, Egypt. Kits for biochemical analysis were purchased from the Gamma Trade Company for Pharmaceutical and Chemicals, Dokki, Egypt.

Rats and Diet:

A total of 25 Sprague–Dawley male rats were obtained from Farm of experimental animals in Helwan, Egypt. Rats weighing 200+20g and were housed in plastic cages and fed on basal diet and water for one week as an adaptation period. Experimental diet and water were offered ad libitum all over the experimental period. Animals were clinically healthy and they randomized and housed in stainless steel wire bottom cages (3 rats /cage) and maintained in air-conditioned room on a 12 h light/ dark cycle at 22+ 2 °C. The basal diet composed of casein (12%), cellulose (5%), vitamins mixture (1%), salts mixture (4%), corn oil (5%) and corn starch (73%). The basal diet formulation was performed according to **A.O.A.C (2006)**.

Experimental design:

A total of twenty five male healthy rats, weighing between (200±20g) were divided into five groups. To induction cirrhosis all rats except control negative were subcutaneous injected by carbon tetrachloride Ccl_4 (which obtained from El- Gomhorria Company, Cairo, Egypt) that diluted by paraffin oil (1:1) {in a dose of 2 ml /kg of body weight of rat}, twice in the week during the experimental feeding period according to the method described by **(Wilfried et al., 1994).** Each group containing 5 rats. Control groups (1&2) negative and positive were fed on basal diet without supplementation. All treated cirrhotic groups (3, 4 &5) were fed on experimental diets as the following:

Group (3): Fed on basal diet containing 0.25% *Spirulina* (algae).

Group (4): Fed on basal diet containing 0.5% *Spirulina* (algae).

Group (5): Fed on basal diet containing 1 % *Spirulina* (algae).

Blood sampling:

At the end of the experimental period (4 weeks), rats were starved for 12 hr. and then sacrificed under ether anesthesia. Blood samples were collected from the aortic vein into clean dry centrifuge tubes and were stored at room temperature for 15 minutes, put into a refrigerator for 2 hour, then centrifuged for 10 minutes at 3000 rpm to separate serum. Serum was carefully aspirated and transferred into dry clean Wasser –man tubes by using a Pasteur pipette and kept frozen at (-20c) till analysis.

Biological Determination:

Determination of food intake, body weight gain and feed efficiency ratio: Food Intake (FI) was calculated every other day, the biological value of the different diets was assessed by the determination of its effect on Body Weight Gain (BWG) and Feed Efficiency Ratio (FER) at the end of the experimental period measured according to **Chapman et al., (1959).**

Biochemical Determination:

Determination of total Lipids, total Lipids (TL) were determined colorimetrically using sulfophosphovanillic mixture according to the method described by **Schmitc (1964)**. Determination of total cholesterol, serum cholesterol was determined according to the enzymatic method described by **Allain et al., (1974)**. Also, determination of triglycerides, the triglycerides in serum were colorimetrically determined according to **Wahlefeld (1974)**. Determination of high density lipoprotein (HDL) cholesterol, the HDL-c was determined according to **Albers et al., (1983**) and determination of very low density lipoprotein (VLDL) cholesterol, the concentration of VLDL-c was estimated according to the Fridewald's equation, VLDL-c = triglycerides / 5. Determination of low density lipoprotein (LDL) cholesterol according to **Fridewald et al., (1972**), low density lipoprotein cholesterol can be calculated as follows: LDL-c = Total cholesterol – (HDL-c) - (VLDL-c).

Determination of liver functions:

Serum activities of aspartate amino transferase AST, alanine amino transferase ALT Alkaline

Phosphatase (ALP) activities were colorimetrically determined according to the method described by **Reitman and Frankel (1957)**.

Determination of kidney functions:

Serum urea nitrogen**,** uric acid**,** creatinine were determined according to the methods described by **Patton and Crouch, (1977), Fossati et al., (1980) and Husdan and Rapoport, (1968)** respectively.

Histopathological Examination:

Specimens from the liver were taken immediately after sacrificing the rats and immersed in 10% neutral buffered formalin. The fixed specimens were then trimmed, washed, and dehydrated in ascending grades of alcohol, then cleared in xylene, embedded in paraffin, sectioned at 4-6 micron thickness and stained with Hematoxylen and Eosin **(Carleton, 1979)** and examined microscopically.

Statistical Analysis:

The obtained data were statistically analyzed according to **SAS, 1996.**

Results and Discussions

Table (1) show effect of different levels of spirulina on food intake, BWG % and FER.

The effect of different levels of spirulina on food intake (FI), body weight gain ratio (BWG %) and food efficiency ratio (FER) were illustrated in table (1). It could be observed that cirrhotic rats (control positive group) had significant decrease in body weight gain (BWG %) and food efficiency ratio (FER) compared with the control negative group. Our findings are similar to the results of **Saber et al., (2011)**.

Moreover, our results observed that all cirrhotic groups fed on basal diet with different levels of spirulina (0.25, 0.5 and 1%) had significant increase in body weight gain (BWG %) and food efficiency ratio (FER) compared with control positive rats. These results are in agreement **Saber et al., (2011)** and **Aldo Ferreira et al., (2010)**. Recently **Cheong et al., (2010)** affirmed that the anti-hypercholesterolaemia mechanisms of spirulina are still not well understood, although some authors suggest that the addition of this alga into the diet diminishes the intestinal absorption of cholesterol as well as the re-absorption of bile acids in the ileum. Thus, they suggest that spirulina can be considered a functional food capable of reducing the levels of cholesterol and consequently preventing atherosclerosis.

Table (2) shows effect of different levels of spirulina on serum total lipid, triglycerides and total cholesterol

As shown in table (2), it could be observed that cirrhotic rats (control positive group) had significant increase in serum total lipid, triglycerides and total cholesterol compared with control negative rats. Similar results were found in the study of **Saber et al., (2011)** who concluded that there was an increase in serum level of cholesterol, triglyceride. Moreover, CCl4 induced hepatic apoptosis. Also, it could be observed that all cirrhotic groups fed on basal diet containing different levels of spirulina (0.25, 0.5 and 1%) had significant decrease in total lipid, triglycerides and total cholesterol compared with control positive group of rats. The best treatments were spirulina (1%) which had lowest values of total lipid, triglycerides and total cholesterol. Our results seem to corroborate the finding of **Kato et al., (1984)** who show a reduction in the levels of serum cholesterol in animals supplemented with spirulina. The results are agreement with **Bashandy et al., (2011)** who reported that Spirulina significantly alleviated the hepatotoxicity induced by HgCl2 and modified the lipid profile through its antioxidant properties. **Table (3) shows effect of different levels of spirulina on serum LDL-C, HDL-C and VLDL-C**

As shown in table (3), it could be observed that cirrhotic rats (control positive group) had significant increase in serum low density lipoprotein LDL, very low density lipoprotein vLDL and decrease in serum high density lipoprotein HDL comparing with control negative. Also, it could be observed that all cirrhotic groups fed on basal diet containing different levels of spirulina (0.25, 0.5 and 1%) had significant decrease in serum low density lipoprotein (LDL) , very low density lipoprotein (vLDL) and increase in serum high density lipoprotein (HDL) compared with control positive rats . The best treatment was spirulina (1%), which had the lowest value of serum low density lipoprotein (LDL), very low density lipoprotein (vLDL) and the highest value of serum high density lipoprotein (HDL). Using an experimental model, **Kato and Takemoto (1984)** submitted rats to a diet rich in cholesterol with and without spirulina supplementation. In this study, the authors observed an increase in total cholesterol levels overall, LDL + VLDL cholesterol and phospholipids in the serum of the group that did not ingest spirulina. However, there was a significant reduction in the levels of these cholesterol fractions when the animals were supplemented with 16% spirulina. Our findings are in line with that of **Saber et al., (2011)** and **Aldo Ferreira et al., (2010)** who concluded that there was an increase in serum level of LDL and HDL. Moreover, CCl4 induced hepatic apoptosis.

Liver is the key organ of metabolism and excretion is constantly endowed with the task of detoxification of xenobiotics, environmental pollutants and chemotherapeutic agents. This disorders associated with this organ are numerous and varied. CCl, a protoxical 4 substance undergoes metabolism in the liver, resulting in the damage of liver cells, which is capable of producing toxic effects at sites far from liver. The C-phycocyanin is an excellent antioxidant

property and scavenging free radicals like superoxide and hydroxyl radicals **(Opoku et al., 2007).**

Liver enzymes activities were used as important biomarkers for detection of hepatotoxic. Serum hepatic marker enzymes (ALT, AST and ALP) were evaluated for hepatotoxicity. The liver is the most sensitive organ to preoxidative damage because it is rich in oxidizable substances. The increment of the oxidative stress on the cells of the liver and the consequent decrease in the antioxidant ability of the cells result in the occurrence of aggressive cellular damage to the liver cells with destruction of their membranes and the release of the enzymes into the blood stream .The more severe the liver damage the higher the release of the liver enzymes **(El-Khayat et al., 2009)**. The increased levels of serum enzyme such as AST and ALT indicate the increased permeability and damage or necrosis of hepatocytes **(Pari and Arumugam, 2008).** The membrane bound enzymes like ALP and γGT are released unequally into bloodstream depending on the pathological phenomenon **(Li and Zhong, 2004).**

Table (4) shows effect of different levels of spirulina on serum liver function AST, ALT and ALP and kidney function Uric acid, Urea and Creatinine

As shown in table (4), it could be observed that cirrhotic rats (control positive group) had significant increase in serum liver function and kidney function parameters (AST, ALT, ALP, uric acid, urea and creatinine) compared with the control negative group. The results are agreement with **Saber et al., (2011)** who concluded that there was an increase in serum level of ALT, AST and ALP. Moreover, CCl4 induced hepatic apoptosis.

Also, it could be observed that all cirrhotic groups fed on basal diet containing different levels of spirulina (0.25, 0.5 and 1%) had significant decrease in serum liver function parameters (AST ,ALT and ALP) and serum kidney function parameters (uric acid , urea and creatinine) compared with the control positive group rats. Similar results were obtained by **Sakr et al. (2010)** in albino rats intoxicated with CCl4. Moreover, the current results are in accordance with those of **Sreelatha et al. (2009)** and **Lodhi et al. (2009)** who reported that liver injury including marked alteration of the entire liver structures with degenerative changes were observed after CCl4 administration. Fatty infiltrations were observed in liver of CCl4 treated rats. In agreement with this result **Qiu et al. (2005)** and **Panovska et al. (2007)** reported that CCl4 caused extensive liver necrosis and fatty changes.

Histopathological examination:

Microscopicall examination of liver of rat in negative control group , showing the normal histological structure photo (1) .While microscopicall examination of liver of cirrhotic rats which fed on basal diet with Subcutaneous injection by Ccl4 (control positive) showing more sever and prolonged degeneration alteration , swelling of hepatocytes Photo (2). In addition, microscopicall examination of liver of cirrhotic rats which fed on basal diet containing spirulina (0.25 and 0.5%) with Subcutaneous injection by Ccl4 showing mild Degeneration alteration and mild swelling of hepatocytes photo (3&4) respectively. While, microscopicall examination of liver of cirrhotic rats which fed on basal diet containing spirulina (1%) with Subcutaneous injection by Ccl4 showing only apparent normal histological structure Photo (5) .

Conclusion:

It could be concluded that Spirulina by different levels (0.25, 0.5 &1%) improve lipids profile and liver functions especially Spirulina by (1%) which has a best significant protective effect against acute hepatotoxicity induced by carbon tetrachloride (Ccl_4) in rats , which may be due to its free radical scavenging effect and its ability to increase antioxidant activity.

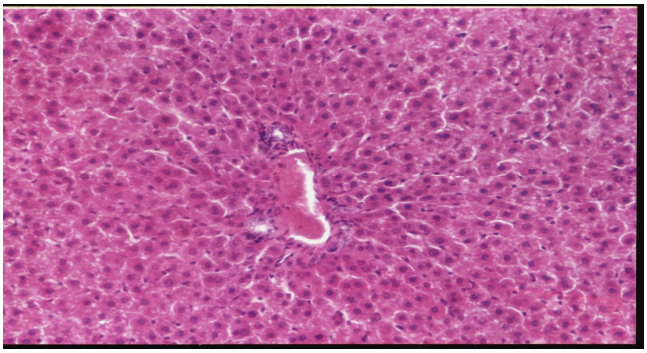

photo (1): Microscopicall examination of liver of rat in negative control group , showing the normal histological structure .

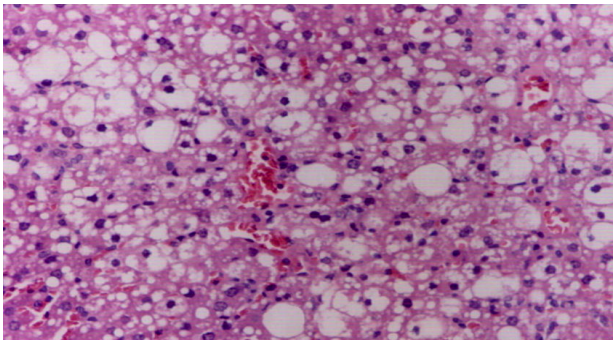

photo (2): Microscopicall examination of liver of rats fed on basal diet with Subcutaneous injection by Ccl4 without treated showing sever and prolonged degeneration alteration , swelling of hepatocytes

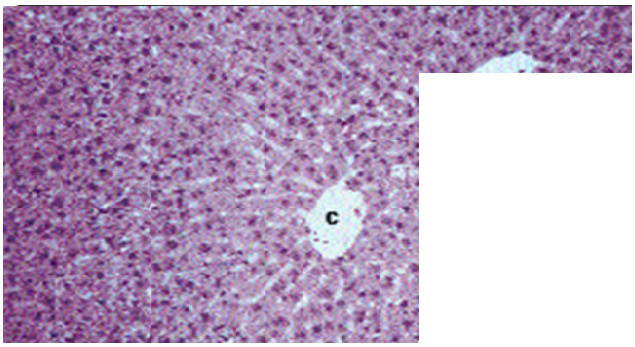

Photo (3) : Microscopicall examination of liver of rats fed on basal diet with Subcutaneous injection by Ccl4 and administrated with Spirulina (0.25%)showing mild Degeneration alteration and mild swelling of hepatocytes

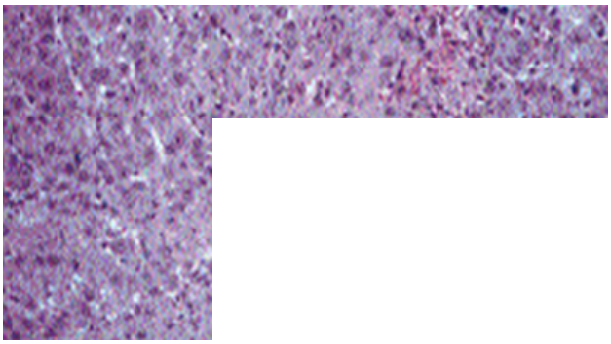

Photo (4) : Microscopicall examination of liver of rats fed on basal diet with Subcutaneous injection by Ccl4 and administrated Spirulina (0.5%) showing mild Degeneration alteration

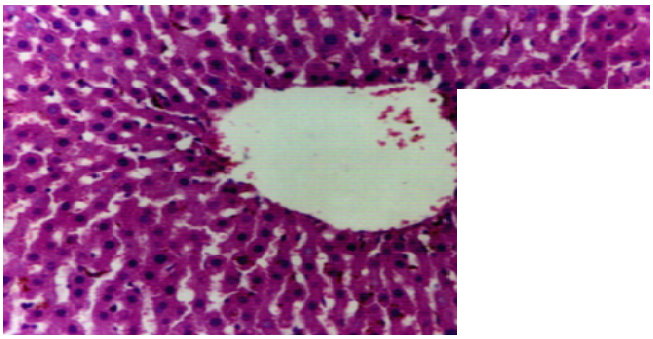

photo (5): Microscopicall examination of liver of rats fed on basal diet with Subcutaneous injection by Ccl4 and administrated with Spirulina (1%), showing apparent normal histological structure .

Table (1): Effect of different levels of spirulina on food intake, BWG % and FER (mean ±SD).

Groups	Food intake g/ day	BWG %	FER
1. Control Negative	21.15	54.13±3.44 a	0.22 ± 0.07 a
2. Control Positive	20.55	34.32±2.66 b	0.16 ± 0.06 b
3. Spirulina (0.25%)	20.57	55.51±2.84 a	0.23 ± 0.05 a
4. Spirulina (0. 5%)	20.79	53.75±2.08 a	0.21 ± 0.04 a
5. Spirulina (1%)	21.03	53.99±2.21 a	0.25 ± 0.03 a

* Values with the same letters indicate non- significant difference (P<0.05) and vice versa.
BWG%: Body weight gain ratio FER: Food efficiency ratio

Table (2): Effect of different levels of spirulina on serum total lipid, triglycerides and total cholesterol (mean ±SD).

	Parameters as Mean ±SD		
Groups	Total lipid (mg/dl)	Triglycerides (mg/dl)	Total cholesterol (mg/dl)
1- Control Negative	325.41±2.15 c	40.43±2.41 c	69.18±2.65 c
2- Control Positive	411.53±2.10 a	70.58±2.63 a	110.21±2.72 a
3- Spirulina (0.25%)	344.55±2.66 b	54.75±1.85 b	79.38±2.81 b
4- Spirulina (0. 5%)	337.67±2.33 b	46.95±2.47 c	74.44±1.96 c
5- Spirulina (1%)	324.19±1.74 c	38.25±2.69 c	71.54±2.03 c

* Values with the same letters indicate non significant difference (P<0.05) and vice versa.

Table (3): Effect of different levels of spirulina on serum LDL-C, HDL-C and VLDL-C (mean ±SD).

	Parameters as Mean ±SD		
Groups	LDL-C(mg/dl)	HDL-C(mg/dl)	VLDL-C(mg/dl)
1- Control Negative	33.33±2.25 c	28.36±1.27 a	7.89±1.63 c
2- Control Positive	72.56±2.48 a	23.57±2.45 b	13.98±1.75 a
3- Spirulina (0.25%)	42.71±1.66 bc	26.75±1.86 a	10.73±1.17 b
4- Spirulina (0. 5%)	37.95±1.84 c	27.92±2.03 a	9.47± 1.69 b
5- Spirulina (1%)	34.01±1.14 c	29.04±1.13 a	7.79±0.80 c

* Values with the same letters indicate non significant difference (P<0.05) and vice versa.

Table (4): Effect of different levels of spirulina on serum liver function AST, ALT and ALP and kidney function Uric acid , Urea and Creatinine (mean ±SD).

Groups	**AST (U/L)**	**ALT (U/L)**	**ALP (IU/L)**	**Uric acid (mg/ dl)**	**Urea (mg/dl)**	**Creatinine (mg/dl)**
1- Control Negative	66.37 ±1.81 c	39.45±0.95c	32.12±2.93 b	2.02 ±0.12 b	21.26 ±0.84 c	0.59 ±0.02 c
2- Control Positive	125.52 ±1.33a	75.57±1.07a	49.24±2.55 a	2.254 ±0.10 a	48.46 ±1.60 a	1.01 ±0.06 a
3- Spirulina (0.25%)	90.78 ±1.45 b	50.29 ±1.59b	34.06±2.47 b	1.97 ±0.15 b	24.66 ±0.88 c	0.63 ±0.02 b
4- Spirulina (0. 5%)	81.88 ±1.67bc	45.51±1.51bc	33.68±2.69 b	1.99 ±0.09 b	29.83 ±0.67 b	0.65 ±0.04 b
5- Spirulina (1%)	76.98 ±1.59 c	42.23±1.53bc	31.40±2.00 b	1.85 ±0.12 b	30.03 ±0.67 b	0.67 ±0.01 b

* Values with the same letters indicate non significant difference (P<0.05) and vice versa.

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