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Effect of Dietary Supplement with Spirulina Platens on the Functional and Histological Changes in the Liver of Mice

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ABSTRACT

Spirulina is unique among blue-green algae, it has a long history of safe use, Spirulina have many benefits for many body systems. The present study deals with the effect of Spirulina Platens that purified from DXN company in Malaysia on the function and histological changes of liver in mice. This was achieved by evaluating the following: 20 mice were divided in to four groups each group has 5 mice: treated groups G1, G2 and G3 (200ug/kg, 100 ug/kg and 50 ug/kg) respectively were orally administration with spirulina compare with control group was administrated normal salin . Body weighs, blood test (haematology and biochenmical parameters) and histological evaluations were studied. The results were showed a significant ($p< 0.05$) increases in Hb, MCHC, MCV WBC and lymphocytes, levels in G1 compare with control. Other hand the results of liver biomarkers such as cholesterol, Triglycerides , AST showed decreased in G1. a significant difference ($p< 0.05$) in B.W in all groups compare with control. In conculsion. The results of the study reflected that Spirulina was a valuable and more effective in treatment and It seems deserves attention as potential natural supplemented.

Keywords: spirulina , diet supplement, liver, biochemical

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INTRODUCTION

Spirulina is a microscopic filamentous cyanobacterium commercially cultivated for food use the name Spirulina indicates the filamentous nature and the spiral or helical shape this name also is used to refer the dried biomass of Arthrospira platensis and defined as an oxygenic photosynthetic bacterium found in fresh and marine waters all over the world. Spirulina grows only in alkaline lakes with an extremely high pH and in large outdoor ponds under controlled conditions, however it is relatively easy to cultivate (Abdulqader et al.,2000). Earlier classification placed Spirulina in the plant kingdom since it has plant pigments and ability to conduct photosynthesis. However now it is included in the kingdom of bacteria based on the better understanding of its genetics, physiology and biochemical properties (Vonshak,2002). Although a large number of Spirulina species are known only three species of Spirulina, namely S. platensis, S. maxima and S. fusiformis were intensively investigated since these species are edible with high nutritional as well as potential therapeutic values (Khan et al.,2005; Karkos et al.,2008). Spirulina has become one of such nutraceutical food with diverse beneficial effects on an array of disease conditions. It has been reported that consumption of Spirulina as diet supplement has health benefits in preventing or managing hypercholesterolemia, hyperglycerolemia, certain inflammatory diseases, allergies, cancer, environmental toxicant, drug-induced toxicities,viral infections, diabetes and other metabolic disease among others(Kulshreshtha et al.,2008).According to the United Nations World Health Organization Spirulina is "the best for tomorrow," and since then it has got popularity recently as a healthy food supplement (Anitha and Chandralekha,2010). Spirulina have many benefits for many body systems, especially immune system, and several toxicological studies have revealed that Spirulina is safe for human use (Salazar et al.,1996; Belay,2002). Thus Spirulina has now become one of the substances that are listed by the use food and drug administration under the category generally recognized as Safe "GRAS" (Tarantino,2003). Several experimental studies have advocated that Spirulina has antiviral, anticancer and immune-supporter properties (Mathew et al.,1995; Hirahashi et al.,2002). Considered as a rich source of protein and vitamins supplement in human diets without any notable side-effects owing to its high (up to 70%) content of protein, besides vitamins especially B12 and pro-vitamin A (β-carotenes), and minerals especially iron (Karkos et al.,2011; Kapoor and Mehta,1993; Simpore et al.,2006).Cyanobacteria have been used as dietary supplements without showing any significant side-effects for several years and Spirulina is cultivated all over the world as a dietary supplement as well as a lunch food and is available as tablet flake or in powder form, it is also used as a fertilizer supplement in the aquaculture, aquarium and poultry feed (Vonshak,2002).

MATERIALS AND METHOD

1. Preparation of spirulina supplement

This study we used supplement of spirulina from DXN company In powder shape and suspened with water in Biology department college of science university of Anbar. The spirulina was obtained from culture collection of DXN It was prepared according to method described by the site of company.

2.2. Experiment animal

The experimental animals using the are mice from the Cancer Research Center in Baghdad. Twenty lab mice weighting about 20-25 g were housed in single cage before two week of experiment and feed with belet and water , the mice were randomly allocated into two main groups namely, the control and treated groups. The control group with five mice (n=5) that was administered with a single dose of normal saline (100µl) and the treated group consists of 15 mice in total which were divided into three sub-groups with five mice for each

group (n=5), (200mg⊠ml (G1), 100mg⊠ml (G2) and 50mg⊠ml (G3).

2.3 .Biochemical analysis

Effectiveness of AST and ALT was estimated according to the method attached to the measurement kit processed by the French company BioMerieux. Cholesterol and triglycerides were measured by the enzymatic method used by French company SYRBIO.

2.4 .Hematology Analysis

Hemoglobin (Hb) was determined , mean corpuscular volume (MCV) , mean corpuscular hemoglobin concentration (MCHC) as described in the method of action (Hillman andAult,2002). Total white cell count (WBC) and differential leukocyte count as reported by(Sood,1985).

2.5. Histological Preparation

The liver tissues samples were cut and trimmed to approximately 0.5 cm3 sizes and fixed in formalin 10% for 48 hours. The fixed samples were placed in plastic cassettes and dehydrated using an automated tissue processor (Leica ASP300, Germany) according to the standard protocol. The processed tissues were embedded in paraffin wax (spain) and the blocks were sectioned at 5 μm thick using a microtome (slee CUT4062,Germany). The tissue sections were mounted on glass slides using a hot plate (Leica HI1220, Germany), followed by the hematoxylin and eosin (H&E) staining (India).

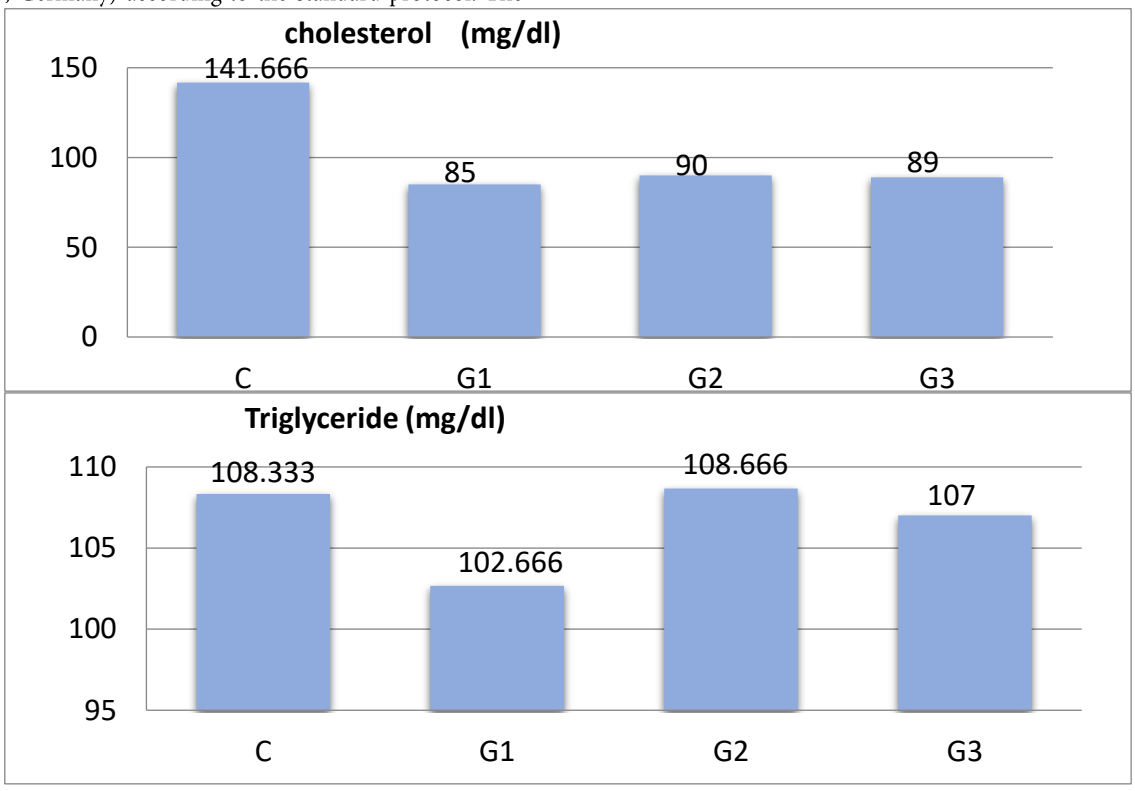
2.6. Statistical Analysis

The Statistical Analysis System –SAS (2012) was used for the analysis of data, and to determine the significance differences between treatments according to the Completely Randomized Design-CRD, and compared the significant differences between the mean by Least Significant Differences (LSD), with a probability level ($p < 0.05$).

.RESULTS3

3.1. Effect of Spirulina on biochemical variables

The results of statistical analysis (Figure 1) showed significant($p < 0.05$) differences. A significant reduction in the level of triglycerides, cholesterol and AST was observed and decreased in the first group (high concentration) at a rate (102.666 mg/dl, 85 mg/dl and 111.666 IU/L), respectively, compared with the control group. Also accompanied by a decrease in the level of ALT enzyme in the third group (low concentration) and reached (355.66 IU/L)



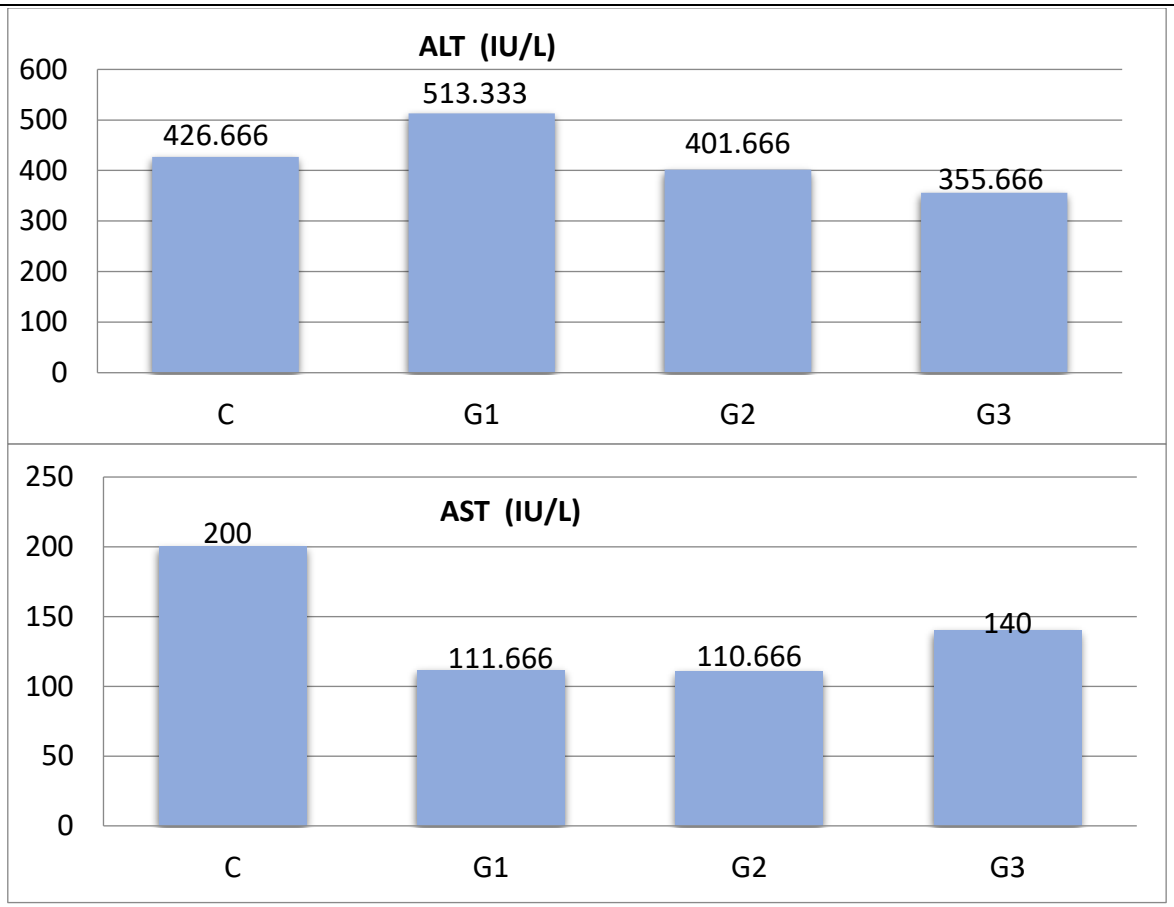
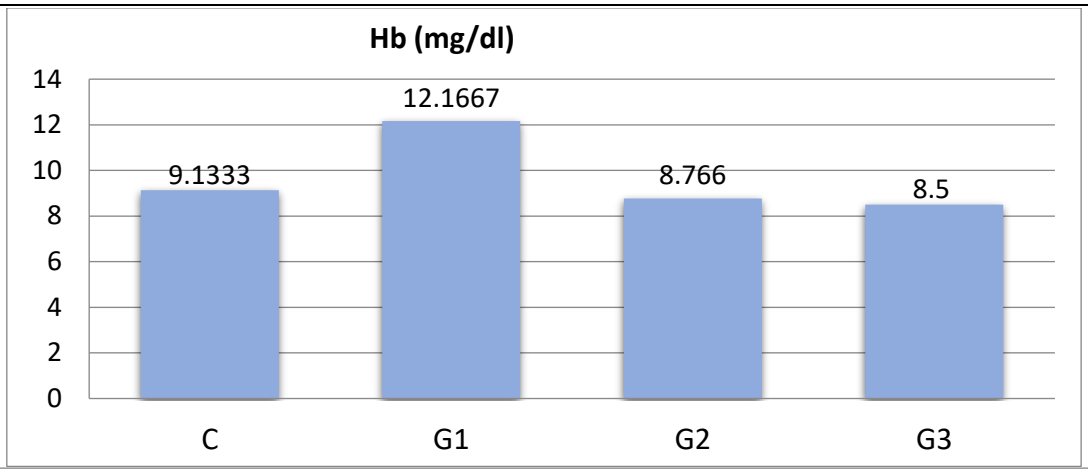
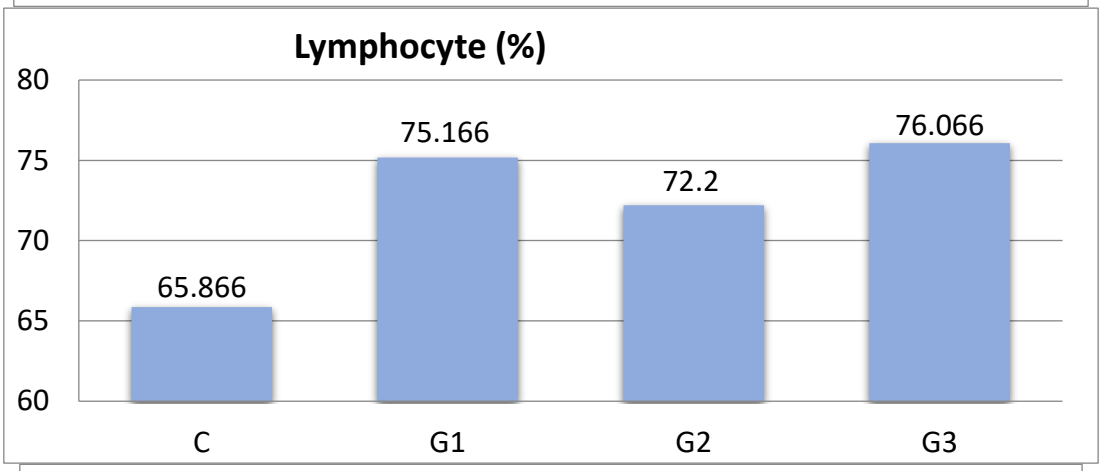


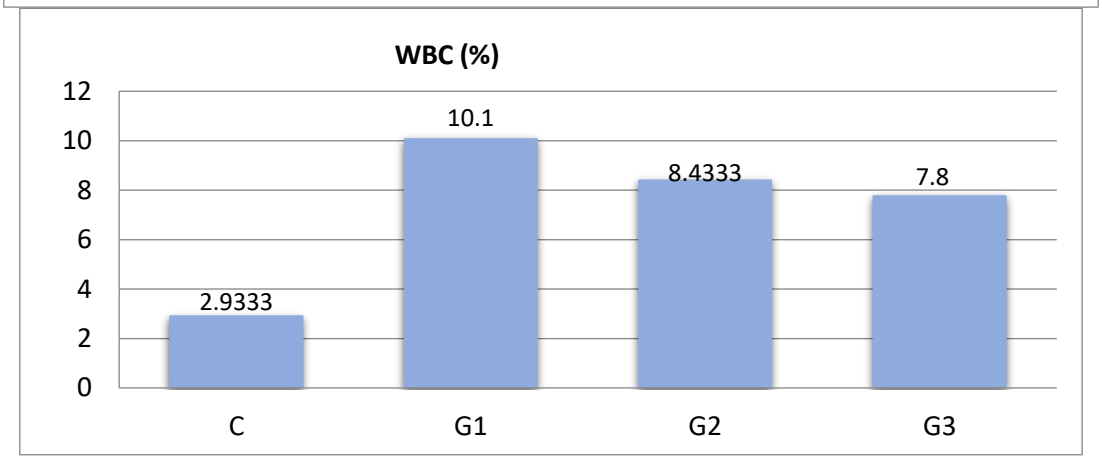
Figure 1.Effect of spirulina on biochemical variables (Triglyceride ,cholesterol,ALT and AST)

3.2 .Effect of spirulina on haematology variables

The results in (Figure 2) showed a significant increase in all variables(MCHC, MCV, Hb, WBC and lymphocytes) and had the highest mean in the first group, reaching (33.266 mg dl,50.33 fl,12.16 mg dl,10.1% and75.166%) respectively , compared with the control group.


Hb (mg/dl)
14
12
10
8
6
4
2
0
9.1333
12.1667
8.766
8.5
C
G1
G2
G3


Lymphocyte (%)
80
75
70
65
60
65.866
75.166
72.2
76.066
C
G1
G2
G3


WBC (%)
12
10
8
6
4
2
0
2.9333
10.1
8.4333
7.8
C
G1
G2
G3

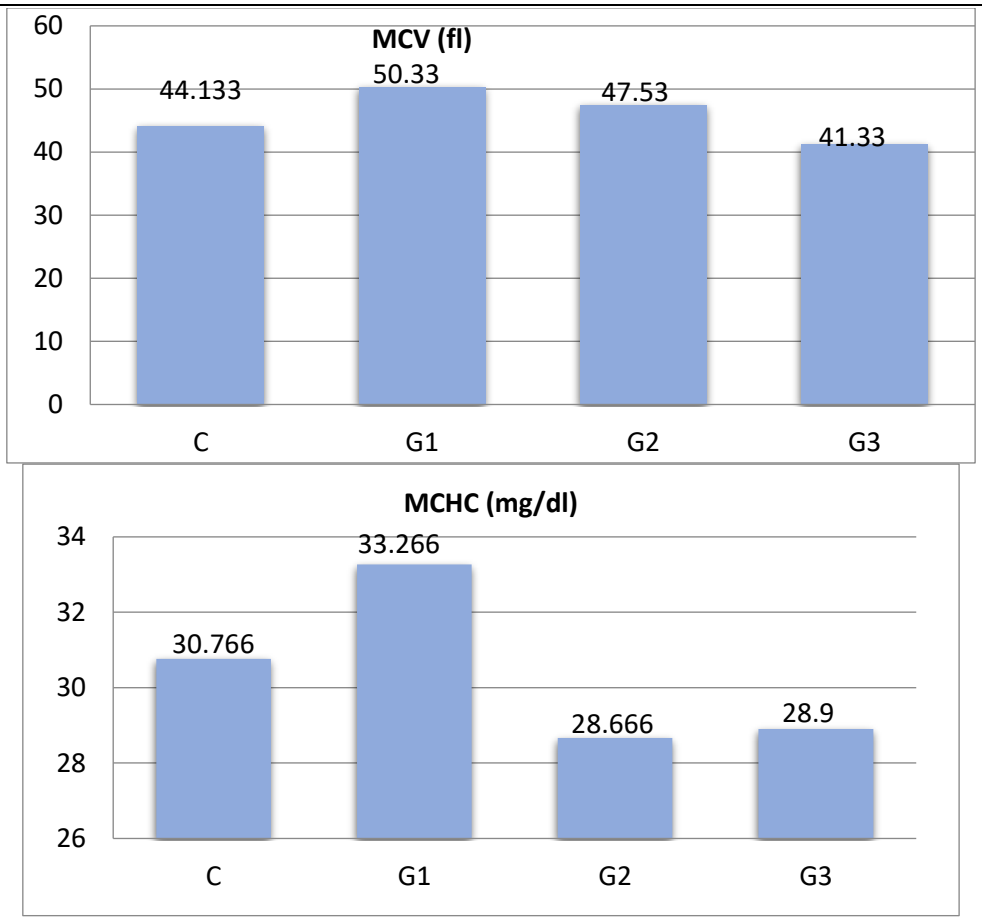


Figure 2. Effect of spirulina on haematology variables (MCHC,MCV,Hb,WBC and lymphocyte)

3.3. Effect of spirulina on body weight

In (Figure 3) showed statistically significant differences ($P < 0.05$) in body weight in all groups compared with the control group.

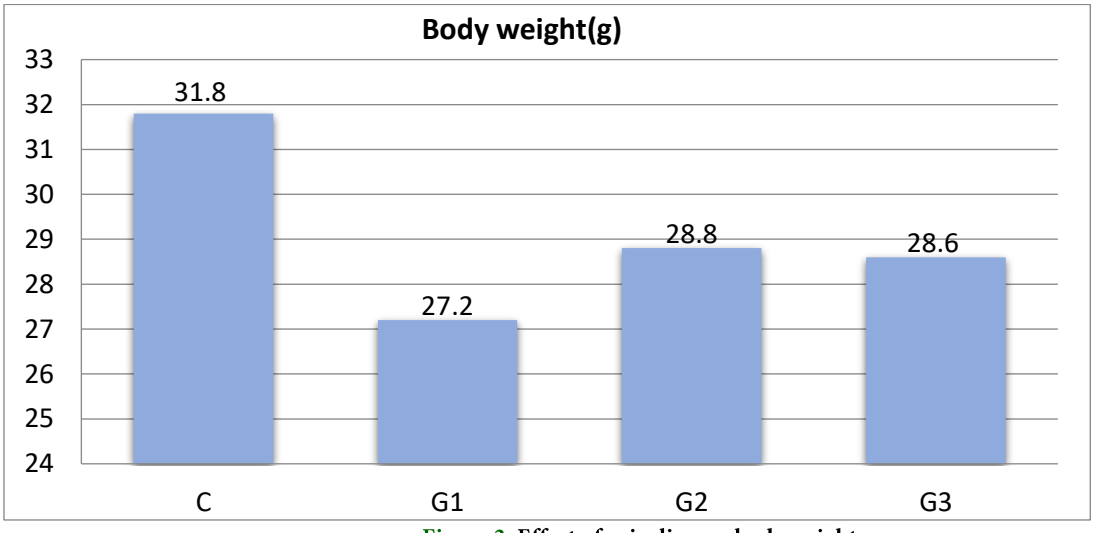


Figure 3. Effect of spirulina on body weight

3.4.Effect of spirulina on Histological examination of liver

The histological sections of the liver showed that the organ is in a normal state in the treated groups. Conversely, in the control group that showed hepatocyte degeneration were higher than other groups using hematoxylin and eosin patches (Figure4).

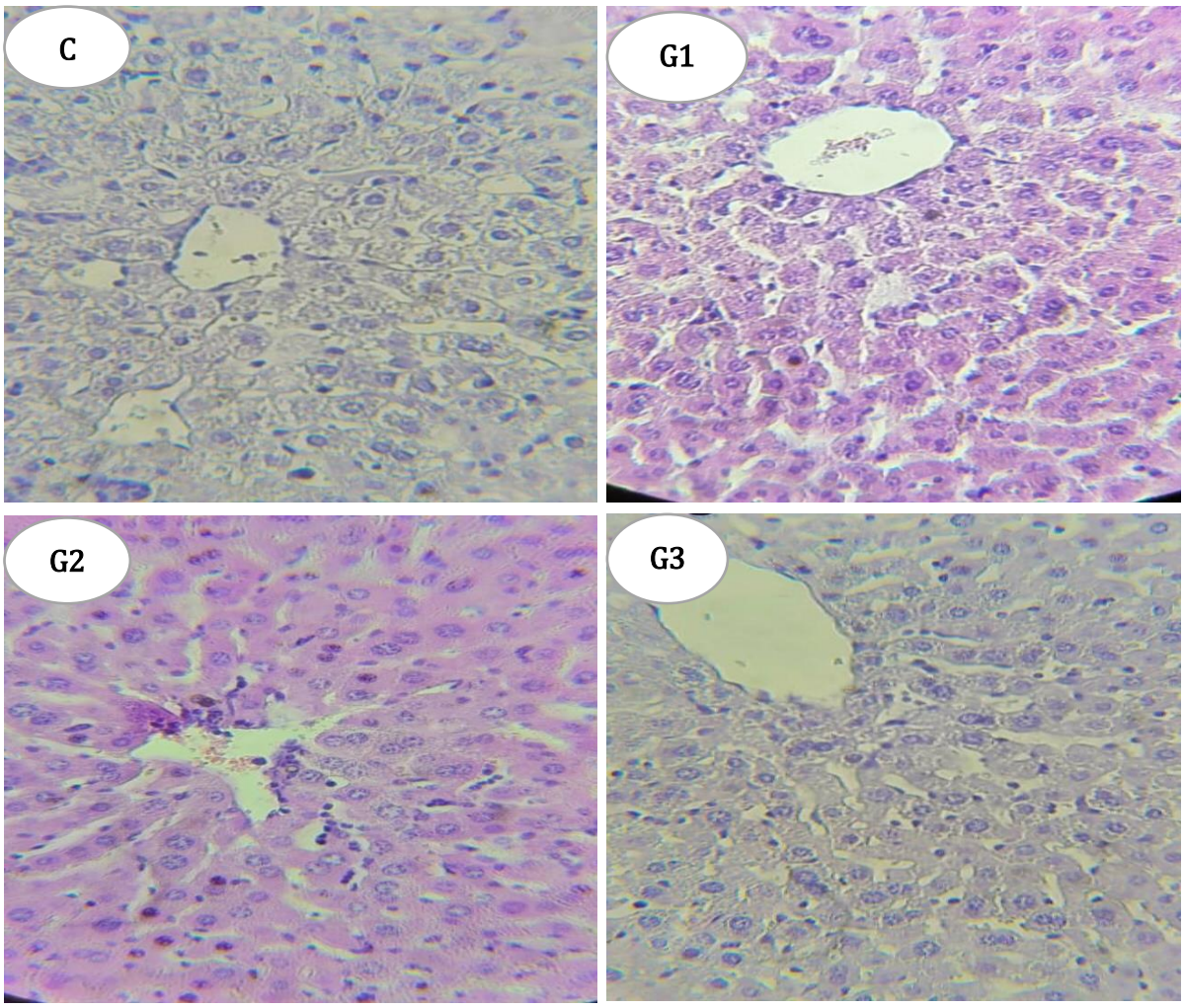


Figure 4. Histological section of the liver in treated groups(G1,G2 andG3), showing normal hepatocyte(NH),central vein (CV) ,sinusoid(Si), and control group showing hepatocyte degeneration (HD). (H&E stain), (100X)

DISCUSSION

Based on the available data we hypothesized that dietary Spirulina supplementation can improve anemia as well as immune function Previous studies have confirmed, spirulina is a powerful stimulant of the immune system, medical scientists have discovered that spirulina not only stimulates the immune system but it actually enhances the body's ability to generate new blood cells and important parts of the immune system, bone marrow stem cells, T cells, natural killer cells, spleen and thymus (Lee,1997). From the results of this study we found significant changes in blood standards. Spirulina commonly used as a dietary supplement is among the most important supplements it modifies the production of cytokines by mononuclear cells of peripheral blood and promotes the production of red cells not surprising given the rich content of flavanoids and sulfa fats (Selmi et al.,2011). The results of this study showed lower levels of functional changes in the liver compared to control. On the other hand, serum lipid profiles changed one month after the treatment of naturally induced hyperlipidemia, Spirulina is able to reduce inflammation as well as antioxidant effects (Gad et al.,2011). Due to the different concentrations of spirulina which led to a significant reduction in total cholesterol and triglycerides, these are consistent with other studies (El-Sheekh et al.,2014). In line with previous results the present results indicated that Spirulina prevented the dietary hypercholesterolemia(Abdel-Daim et al.,2016). Several reports also suggested that Spirulina could have a beneficial effect in the prevention of hypercholesterolemic cardiovascular diseases, some studies have presumed that its high C-phycocyanin content inhibits pancreatic lipase activity and Its C-phycocyanin content is also presumed to act together with glycolipid hemoglobin (Hb)-2, leading to a decrease in cholesterol absorption(Ferreira-Hermosillo et al.,2010). Published scientific studies in Japan and India showed several grams of Spirulina daily can lower cholesterol. and suggest will reduce serum LDL (Low Density Lipoproteins) and raise HDL (High Density Lipoproteins). This study demonstrated a decrease in body weight of the treated groups compared with control, and these findings support a lower level of cholesterol and fat. This is consistent

with what human studies in Germany have found to have a weight-loss effect along with lowering cholesterol. All these studies indicate that spirulina is a useful supplement for cardiovascular health and for lowering cholesterol (Abdel-Daim et al.,2016). Our results showed a decrease in the level of enzymes AST and ALT. The enzymes in our bodies digest our food, cut, paste, and repair our DNA and provide energy to critical body systems. In contrast spirulina administration had beneficial effects on hepatic equilibrium and significantly reduced liver function parameters(Aissaoui et al .,2017).

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