Tikrit Journal of Pure Science Vol. 30 (1) 2025

DOI: *https://doi.org/10.25130/tjps.v30i1.1698*

Tikrit Journal of Pure Science

ISSN: 1813 – 1662 (Print) --- E-ISSN: 2415 – 1726 (Online)

Journal Homepage: https://tjpsj.org/

Effect of Feeding with Spirulina and Folic Acid on Some Biochemical Parameters and Histological of Laboratory Rats (Albino Rats) with Induced Anemia

Zyad Tariq Hussein, Sami Khudhur Saeed

Department of Food science, College of Agriculture, Tikrit University, Tikrit, Iraq

Received: 14 Jul. 2024 **Received in revised form:** 25 Aug. 2024 **Accepted:** 8 Sep. 2024

Final Proofreading: 20 Sep. 2024 **Available online:** 25 Feb. 2025

ABSTRACT

The current study aims to evaluate the preventive effects of extracts of spirulina and folic acid in treating phenylhydrazone-induced anemia and to study some biochemical and histological alterations on blood parameters, blood lipid profile, The liver and renal functioning has been used and divided into five groups of 5 animals per group at random in plastic cages. The groups were as follows. The first group was the health control group fed the standard diet. The second group was the anemia-induced group, which was injected subcutaneously with Phenylhydrazine (PHZ) (20 mg/kg body weight for 48 hours). The third group received spirulina extract at a dosage of (100 mg/ml). The fourth group received folic acid extract at a dosage of (40 mg/ml). The fifth group received extracts of spirulina and folic acid at a combined concentration of (20 mg/ml) of folic acid and (50 mg/ml) of spirulina. The dose was administered given orally. The study found that PHZ **(**$C_6H_5NHNH_2$**)**injection led to a substantial decrease ($P \leq 0.05$) compared to the healthy control group in liver enzymes ALT, AST, and affected the blood lipid profile, as the values of total TC, TG, LDL, and VLDL levels all rose, but HDL levels decreased as compared to the values in the healthy control group. Dosing the rats previously injected with PHZ with spirulina and folic acid resulted in a beneficial effect and improvement in liver enzymes, as the values of ALT and AST reduced while the blood lipid profile improved.

Keywords: Spirulina, Folic Acid, Liver enzymes, Lipid profile.

zyad.t.h0240@tu.edu.iq

Name: **Zyad Tariq Hussein** **E-mail:** zyad.t.h0240@tu.edu.iq

تأثير التغذية بالسبيرولينا وحامض الفوليك على إنزيمات الكبد ومرتسم دهون الدم في الجرذان المختبرية نوع البينو المصابة بفقر الدم المستحث

زياد طارق حسين، سامي خضر سعيد

قسم علوم الاغذية، كلية الزراعة، جامعة تكريت، تكريت، العراق

الملخص

تهدف الدراسة الحالية إلى تقييم التأثيرات الوقائية لمستخلصات السبيرولينا وحمض الفوليك في علاج فقر الدم الناجم عن الفينيل هيدرازون ودراسة بعض التغيرات الكيميائية الحيوية والنسيجية على معايير الدم ومستوى الدهون في الدم ووظائف الكبد والكلى وقد تم استخدام خمس مجموعات من 5 حيوانات لكل مجموعة بشكل عشوائي في أقفاص بلاستيكية وكانت المجموعات على النحو التالي. المجموعة الأولى كانت مجموعة التحكم الصحية التي تغذت على الغذاء القياسي. المجموعة الثانية كانت مجموعة فقر الدم الناجم عن ذلك والتي تم حقنها تحت الجلد بفينيل هيدرازين **(PHZ)** (20 ملغ / كغ من وزن الجسم لمدة 48 ساعة). المجموعة الثالثة تلقت مستخلص السبيرولينا بجرعة (100 ملغ / مل). المجموعة الرابعة تلقت مستخلص حمض الفوليك بجرعة (40 ملغ / مل). المجموعة الخامسة تلقت مستخلصات السبيرولينا وحمض الفوليك بتركيز مشترك (20 ملغ / مل) من حمض الفوليك و (50 ملغ / مل) من السبيرولينا. تم إعطاء الجرعة عن طريق الفم. وتوصلت الدراسة إلى أن حقن **$C6H5NHNH_2$ (PHZ** أدى إلى انخفاض كبير (**$P \leq 0.05$**) مقارنة بمجموعة التحكم الصحية في إنزيمات الكبد **ALT** و**AST**، وأثر على ملف الدهون في الدم، حيث ارتفعت قيم مستويات **TC** و**TG** و**LDL** و**VLDL** الكلية، ولكن انخفضت مستويات **HDL** مقارنة بالقيم في مجموعة التحكم الصحية. أدى إعطاء الفئران المحقونة مسبقًا بـ **PHZ** مع سبيرولينا وحمض الفوليك إلى تأثير مفيد وتحسن في إنزيمات الكبد، حيث انخفضت قيم **ALT** و**AST** بينما تحسن ملف الدهون في الدم.

1. INTRODUCTION

Anemia is a widespread health problem all over the world. The most important symptoms of the disease are feeling tired, thin, and stressed, especially oxidative stress and psychological pressure. Psychological stress may contribute to oxidative stress in the body, which is represented by a change in the balance between oxidants and antioxidants. An increase in the values of TC, TG, LDL, VLDL, and HDL has been observed, in addition to a change in some blood parameters, such as an increase in the number of PLT [(1)]. The term functional foods was first coined in Japan in the mid-1980s to describe processed foods that contain substances that aid in various biological functions while also being healthy. Japan is the only country that has developed a unique regulatory clearance process for functional foods. These foods are called Food for Specified Health. Uses (FOSHU) [(2)]. Functional foods play an important role in disease prevention and health promotion, and several organizations have proposed definitions for this new and emerging field of food and nutrition science. The Food and Nutrition Board of the Institute of Medicine (IOM/FNB, 1994) defined functional foods as “any food or dietary component that may provide a health benefit beyond traditional nutrients”. Studies have shown that dietary supplements that provide multiple vitamins and minerals can fill nutritional gaps for pregnant women and those with anemia and have been shown to be a safe and cost-effective intervention to reduce adverse pregnancy and birth outcomes. The most important of these foods rich in vitamins and minerals are:

1.1. Spirulina plants

The genus Spirulina belongs to the blue-green algae and possesses a wide range of colorful and biologically active components such as phycocyanin, carotenoids, and chlorophyll. Spirulina is a microalga that contains nutrients that have been employed as a functional food as well as medicinal and pharmacological materials[3]. Its chemical composition includes proteins (55 % - 70 %), carbohydrates (15 % - 25 %), essential fatty acids (18 %), vitamins, minerals and pigments such as carotenoids, chlorophyll A and phycocyanin. Because dyes are used in the food and cosmetic industries, spirulina is regarded as a great diet with low toxicity and immunological and antiviral characteristics. It also functions as a potent antioxidant [4]. Spirulina has been demonstrated in studies to be useful in the treatment of allergies, anemia, cancer, liver toxicity, viral and cardiovascular disorders, hyperglycemia, hyperlipidemia, immunodeficiency, and inflammatory conditions. Many of these activities are linked to Spirulina itself or some of its components, which include omega-3 or omega-6 fatty acids, beta-carotene, alpha-tocopherol, phycocyanin, and phenolic compounds[5]. The antioxidant, immunomodulatory, and anti-inflammatory activities of this microalgae (Spirulina) may play an important role in human health [6].

1.2. Folic Acid

1- Folic acid (FA) is one of the types of B vitamins, vitamin B9, and is considered one of the most important vitamins that are beneficial to the human body in general. Its chemical formula is $C_{19}H_{19}N_7O_6$[7]. The discovery of folic acid was the product of the hard labor of Lucy Wills, a medical researcher who graduated from Cambridge University with a degree in botanist and geologist in 1930 and worked in India after she became particularly interested in the problem of acute anemia among poor textile workers. The industrial synthesis cycle for folic acid was developed in 1945. Since then, studies have shown folic acid's capacity to produce a prompt hematologic response in many cases of macrocytic anemia, save those that were determined to be "harmful". Folic acid is a water-soluble B vitamin that can be found naturally in foods such as green leafy vegetables, asparagus, broccoli, citrus fruits, legumes, dried grains, whole grains, yeast, beans, liver, and other organ meats. Folic acid, the synthetic form of this vitamin used commercially in supplements and fortified meals, has been considered an optimum nutritional element for cancer prevention and anemia[8]. In humans, folic acid is obtained from the diet and is transported across the brush border membrane of enterocytes into the jejunum after absorption. It is released into the portal circulation and absorbed by the liver, which is the main regulator of folate balance within the plasma [9]. Folic acid is used to manage and treat megaloblastic anemia and folate deficiency anemia. One of the most essential reasons for taking folic acid is to consider the development of the central nervous system. Women who wish to become pregnant should take folic acid supplements to lower their chance of neural tube abnormalities. Folic acid can help guard against tumors in ulcerative colitis, prevent cervical dysplasia, treat vitiligo, restore blood formation in macrocytic anemia caused by folate deficiency, and strengthen the resistance of the gums to irritants, thereby lowering inflammation [10].

2. MATERIALS AND METHODS

2.1. Preparing the feed for the experimental animals

The standard feed, which represented the basic food used in the experiment, was prepared according to[11] Table (1). The ingredients were mixed well for homogeneity, then moistened with clean distilled water to form a dough, after which it was cut into discs and dried at a temperature of 60°C. The feed was prepared weekly as needed.

Table 1: Standard feed ingredients g/kg as reported in [11]

Material	Cellulose	Vitamin Mixture	Mineral Mixture	Starch	Casein	Sunflower Oil	Glucose
Weight/g	50	5	50	536.5	158.5	100	100

2.2. Preparation of experimental animals in the laboratory

The experiment took place in the College of Veterinary Medicine's animal house at Tikrit University, where adult male albino rats aged between 8 and 9 weeks weighing between (140 and 159 g) were used. A veterinarian examined the animals to ensure that they were free from diseases, in addition to a one-week follow-up to ensure Physical activity and health indicators. Suitable conditions were provided with a temperature of about (25 °C) with ventilation and humidity at (50 %), and a light-to-dark period of (12:12) hours. The animals were distributed in plastic cages with metal mesh covers with dimensions of (19 × 21 × 25 cm). The floor was covered with sawdust, taking into account the aspect of cleanliness that included changing the sawdust twice a week and sterilizing the cages with disinfectants. The animals were given open access to water by a customized container attached to the cage. The animals were dosed according to the groups required to be dosed with spirulina and folic acid at a dose every (24 hours) orally using a gastric tube Gavage by dissolving the specified dose for each animal with (2 ml) in the case of Folic acid and (2 ml) in the case of spirulina.

2.3. Experimental design

This experiment used 25 male albino rats, five animals per group. It lasted for five weeks, from September 25, 2023, to November 2, 2023, including two weeks to reach the state of hyperemia and four weeks for treatment.

The experimental animals were randomly assigned to five groups, each with its diet, as follows:

1- The first group (positive control T-) consisted of rats fed a regular diet.

2- The second group (negative control T+): This group of rats was fed a standard diet, and anemia was induced in them by injecting PHZ at a concentration of (20 mg/ml) subperitoneally for two consecutive days.

3- The third group (T1): The group of rats fed on a standard diet, anemia was induced in them by injecting PHZ and dosed with a concentration of (100 mg/ml) of spirulina.

4- The fourth group (T2): The group of rats in which anemia was induced by injecting PHZ and fed on a standard diet and dosed with a concentration of (40 mg/ml) of folic acid.

5- The fifth group (T3): The group of rats in which anemia was induced by injecting PHZ and dosed with (20 mg/ml) folic acid and (50 mg/ml) spirulina.

2.4. Samples

2.1 −Collection of blood samples

After the 30-day trial, the animals were fasted for (24 hours) and sedated with chloroform, and blood samples were collected via a heart puncture. The drawn blood was divided into two parts, one of which was placed in tubes containing the anticoagulant (EDTA) for the purpose of directly conducting the CBC (Complete Blood Count) tests. In contrast, the remaining part was placed in gel tubes. A centrifuge was used at a speed of (3000 rpm) for (10 minutes), after which the serum was separated from the other components and then placed in Eppendorf Tubes and stored under freezing at a temperature of (-20 °C) until the required[12]. Determination of Triglycerides (TG) Concentration in blood serum

The level of triglycerides in blood serum is estimated using the ready-made test kit manufactured by the Italian company Giesse Diagnostics based on the method [13]. Determining Total Cholesterol Concentration in Blood Serum The concentration of cholesterol in blood serum is measured according to this method, which he came up with. The total cholesterol concentration was calculated according to [14]. Determination of high-density lipoprotein cholesterol (HDL) concentration in blood serum[14].

3. RESULTS AND DISCUSSION

3.1. Study of the values of liver enzymes in the blood serum

The results of the experiment showed, as shown in Table (2), a significant increase in liver enzymes ALT and AST in the positive control group with anemia by injection with PHZ compared to the healthy control group. Injecting the experimental rats with PHZ led to a significant increase in liver enzymes. Any injury to the liver leads to an increase in ALT, AST values as a result of the toxicity caused by PHZ. PHZ injection leads to the accumulation of excess iron in the liver because of increased oxidation processes and the production of free radicals generated and accumulated because of the exposure of animals to PHZ toxicity, which leads to increased degeneration of hepatic cells and inflammatory cells and, consequently led to an increase in liver enzymes AST, ALT[15]. Through the results of previous and current studies, it was shown that oral administration of spirulina in the TI group and folic acid in group T2 and the T3 group, spirulina and folic acid had a positive effect and contributed to improving the AST and ALT values, as the AST values were 119 in T1, 149 in T2 and 116 in T3 compared to the positive control group with anemia T+, where its value was 160 and the healthy control group T-, which had a value of 128, while the ALT values were respectively 57, 70 and 49 in the T1, T2 and T3 groups compared to the negative control group, which had a value of (71 mIU/ml). The results agreed with[16], who found that exposing experimental animals to deltamethrin, a toxic chemical compound used as an insecticide, led to increased liver toxicity and increased liver enzyme values because of oxidative stress resulting from free radicals. It was shown that treating rats with spirulina at different doses reduced liver injury and improved liver enzyme levels. This is due to the protective effects of spirulina, which contains antioxidants and active ingredients such as C-phycocyanins, B-carotene, vitamins, minerals, proteins, fats, and carbohydrates. The results also agreed with[17], who explained that exposing rats to acetamiprid, a toxic insecticide, led to increased liver toxicity and increased liver enzymes. They showed that taking folic acid was able to restore liver enzyme levels to semi-normal levels.

Table 2: Effect of treatments with Spirulina and Folic Acid on the activity of liver enzymes in the blood serum of male rats in which anemia was induced.

Group Treatments	Parameters	
	A S T (mIU/ml)	A L T (mIU/ml)
T- Healthy Control	128.50b±0.86	51.50b±4.90
T+ Infected control	160.00a±5.19	71.5a±0.86
T1(Spirulina)	119.00b±1.73	57.50ab±9.52
T2(Folic Acid)	149.50a±6.63	70.50a±0.86
T3(Spirulina +Folic Acid)	116.00b±9.23	49.00b±2.30

Different lowercase letters in the same column indicate significant differences between the rates at a significance level of (± 0.05)

The results of our study agreed with [18], who also found that taking folic acid in different doses had a positive effect in restoring liver enzyme levels to normal levels and the best result was at the highest dose (75 µg/kg). It was also concluded that fluoride led to liver poisoning through oxidative stress, which led to increased secretions of liver enzymes, which are considered an indicator of liver poisoning. It was explained that the use of folic acid was able to reduce liver enzymes, and many studies have proven that folic acid has a strong effect in protecting the liver and resisting oxidative stress resulting from drugs and toxic substances, which is what our current study has concluded.

3.2. Study of the effect of spirulina and folic acid treatments on the blood lipid profile in rats with induced anemia

The results showed that oral administration of both spirulina (T1) caused a significant triglyceride (TG), cholesterol (TC), and low-density lipoproteins (LDL) lower in the spirulina-treated group than in the infected group, with values of (105, 73, and 21 mg/dL), respectively. Also, there was a considerable decrease in low-density lipoproteins (VLDL). Where its value was (22 mg/dl), while the group dosed with folic acid (T2) also had a decrease in the level of triglycerides TG, total cholesterol TC, and low-density lipoproteins LDL compared to their value in the infected group. The results were (111, 73, and 32 mg/dl), and the level of very low-density lipoproteins was (21 mg/dl). As for the group (T3) dosed synergistically with spirulina and folic acid, the results for the level of triglycerides TG, total cholesterol TC, and low-density lipoproteins LDL compared to their value in the infected group were (106, 74, and 31 mg/dl). The level of very low-density lipoproteins was (21 mg/dl). Table (3) shows the values of High-Density Lipoprotein (HDL) which had a significant increase at the probability level ($P \leq 0.05$) in groups T1, T2 and T3 and were (19, 20, and 22 mg/dl) respectively compared to the infected control group which was (13 mg/dl). The results agreed with[19], who found a positive role for spirulina in improving the levels of blood lipid profile as it was able to reduce the level of triglycerides TG, total cholesterol TC, and LDL as well as the level of very low-density lipoproteins and increase the values of High-Density Lipoprotein (HDL) [20]. Spirulina has also been shown to lower triglycerides (TG), total cholesterol (TC), low-density lipoproteins (LDL), and very low-density lipoproteins (VLDL) while increasing high-density lipoproteins (HDL). This is due to the ability of spirulina to bind cholesterol with bile salt metabolites and reduce the solubility of cholesterol as well. This effect may be due to the presence of linoleic acid in spirulina, which prevents the accumulation of fats and cholesterol in the human body. These are the same results obtained in our current study. The results agreed with[21], who explained the ability of spirulina to reduce blood fats due to its antioxidant properties, especially ascorbic acid, which was found to have a significant role in reducing blood fats by preventing the oxidation of LDL, as its oxidation leads to increased cholesterol deposition in the lining of blood vessels.

Table 3: Effect of treatments with Spirulina and Folic Acid on blood fat levels in the serum of male rats in which anemia was induced.

Group treatments	Parameters				
	TG mg/dl	TC mg/dl	HDL mg/dl	LDL mg/dl	VLDLmg/dl
Healthy Control T-	110.50a±3.72	69.50a±50	15.00bc±00	32.40ab±2.77	22.10a±0.75
Infected control T+	113.50a±0.28	77.50a±1.44	13.50c±0.28	36.30a±0.51	22.70a±0.57
T1(Spirulina)	105.00a±4.61	57.50b±50	19.00ab±2.30	21.30b±4.56	22.20a±0.34
T2(Folic Acid)	111.00a±1.73	73.00a±0.57	20.00a±1.73	32.00ab±3.23	21.00a±0.92
T3(Spirulina+Foloc Acid)	106.00a±4.04	74.50a±4.33	22.00a±0.57	31.30ab±5.71	21.20a±0.80

Different lowercase letters in the same column indicate significant differences between the rates at a significance level of ±0.05

indicated that folic acid has a positive and protective role in atherosclerosis and is considered an effective antioxidant and suppresses free radicals caused by homocysteine, as it helped reduce the level of TG, cholesterol TC, and low-density lipoproteins, as well as the level of very low-density lipoproteins. They also found [22] that folic acid has a protective effect against the harmful effects of infrared rays that lead to DNA damage. The study also indicated the control of folic acid over suppressing free radicals resulting from radiation and a protective effect against oxidative stress because of reducing the level of triglycerides and cholesterol. These are the same results we obtained in our current study, which showed that folic acid had a role in improving the blood lipid profile.

3.3. Histological changes of rats induced with anemia by injection of PHZ

the negative control group (T+) of rats with PHZ-induced anemia as shown in Figures (1, 2 and 3) showed that the liver tissue contained many degenerated hepatocytes, and many cells separated from other cells and widespread blood sinusoids continuous with the central vein in addition to their ulceration (congestion of blood vessels) and Kover's cells that were found in many of those sinusoids. It was also found that the liver tissue contained degeneration and atrophy in the rows of necrotic hepatocytes and around them the large-cavity blood sinusoids containing a few Kover's cells and some sloughed hepatocytes [23]. pointed out that drugs and chemicals (glucosamine) had a negative effect on liver tissue compared to the control group, in which liver tissue and kupffer cells were normal.

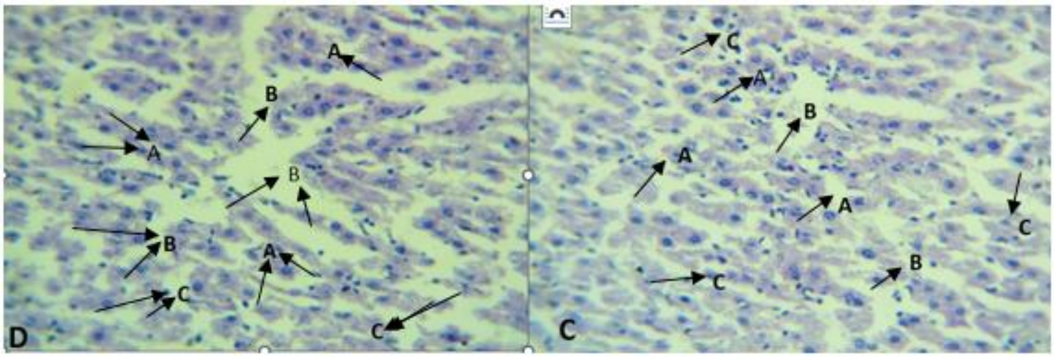

Fig. 1: (A) section of the liver tissue of the infected control group (T+) rats, showing the following: Image (C) Liver tissue Degeneration and disintegration of hepatocytes (A) Expansion of blood sinusoids (B) Kupffer cells (C) (H2 and E x40). Image (D) Degeneration and atrophy of hepatocytes (A) Expansion of the sinusoids (B) Degeneration of hepatocytes into the sinusoidal cavity (C) (H2 and E x40). Harris Hematoxylin & Eosin stain was used according to the method of Luna (1968), and the tissue sections were stained according to the method of Humason (1997).

Histological changes in the liver of rats fed the standard diet and dosed with (100 mg/ml) of spirulina The liver tissue of the group of rats fed the standard diet and dosed with (100 mg/ml) of spirulina (T1) contained rows of hepatocytes, each row consisting of two layers of polygonal cells with dark spherical nuclei, rows or chromatites, and between the rows of cells were found blood sinuses containing a specific number of Kover cells. The hepatic lobule also contained the central vein containing a limited amount of decomposed blood, and the vein was surrounded from the outside by numbers of white blood cells. The lobule also contained groups of hepatocytes closely packed together with prominent nuclei. Around the hepatocytes were found blood sinuses containing some Kover cells, as well as congestion of the sinuses, dilation of blood vessels, expansion in the portal areas, and infiltration of mixed inflammatory cells around necrotic hepatocytes. The results agreed with found, who indicated Histological examination of rats orally dosed with (300 mg/ml) of spirulina resulted in sinus congestion, vasodilation, portal tract expansion, and infiltration of inflammatory cells in the liver.

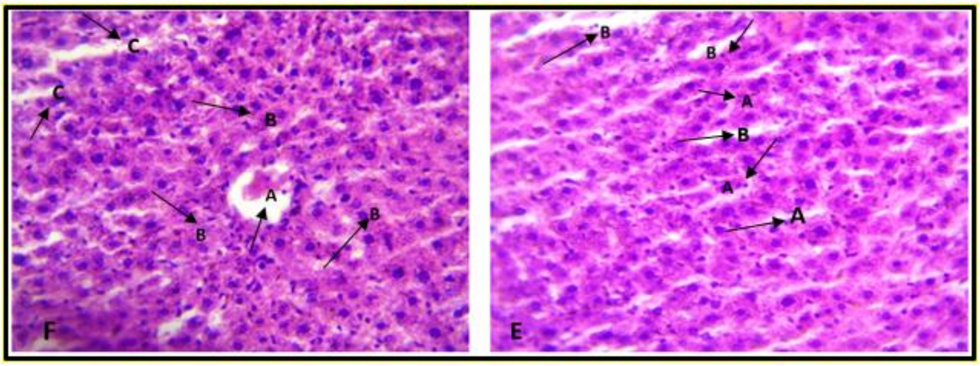

Fig. 2: Section of the tissue of rats fed the standard diet and dosed with 100 mg/ml of spirulina (T1) showing the following: Image (E) Liver tissue Polygonal hepatocyte rows, each row composed of two adjacent cells (A) Weak dilated blood sinusoids (B) (H2 and E x40). Image (F) Central vein with limited blood clot (A) Groups of dense hepatocytes containing prominent dark-pigmented nuclei (B) Blood sinusoids (C) (H2 and E x40). Harris Hematoxylin & Eosin stain was used according to the method of (24), and the tissue sections were stained according to the method of (25) , Histological changes in the liver of rats fed on standard diet and dosed with 40 mg/ml of folic acid (T2)

The liver lobule contained the central vein devoid of blood and the vein lined with some endothelial cells supported by some Koffer cells, the sinusoids appeared as narrow cavities and the hepatocytes were found in an enlarged form where they were found compact with each other and contained a nucleus and some of them were two nuclei in the cytoplasm of the same cell. The central vein in the center of the hepatic lobule contained clumps of blood with the presence of haemoglobin and the branches of the vein were continuous with the cavities of the narrow blood sinusoids containing limited numbers of Koffer cells, the hepatocytes were found in an excessively large form with the presence of vacuolization in the cytoplasm of many hepatocytes in addition to the presence of enlargement in their nuclei.

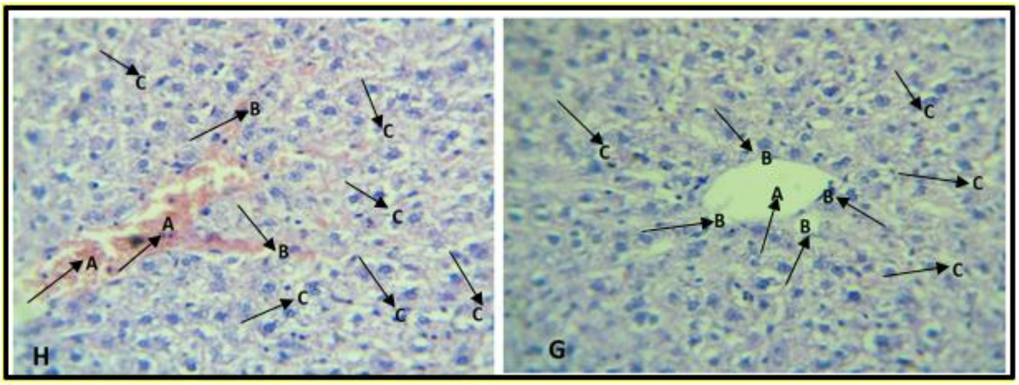

Fig. 3: Section of the liver tissue of rats fed on the standard diet and dosed with folic acid at a concentration of 40 mg/ml (T2) showing the following: Image (G) Liver lobule, central vein devoid of blood (A) lined with endothelial cells (B) Hepatocyte hyperplasia (C) (H2 and E x40). Image (H) Central vein with blood clot with hemosorphin (A) Sinusoids blood (B) Hepatocyte hyperplasia with cytoplasmic rupture (C) (H2 and E x40). Harris Hematoxylin & Eosin stain was used according to the method of (24), and the tissue sections were stained according to the method of (25).

Conflict of interests: The author declared no conflicting interests.

Sources of funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author contribution: Author contributed in the study.

REFERENCES

1. Hamza MA, Abdulla IT, Hamza A. Effect of oxidative stress on lipid profile and blood parameters to a sample of students at university of Zakho during exames. Tikrit J Pure Sci. 2018;23(1):78-82. https://doi.org/10.25130/tjps.v23i1.483

2. Cencic A, Chingwaru W. The role of functional foods, nutraceuticals, and food supplements in intestinal health. Nutrients. 2010;2(6):611-25. https://doi.org/10.3390/nu2060611

3. Gabr GA, El-Sayed SM, Hikal MS. Antioxidant activities of phycocyanin: A bioactive compound from Spirulina platensis. Journal of Pharmaceutical Research International. 2020;32(2):73-85. https://doi.org/10.9734/jpri/2020/v32i230407

4. Alvarenga RR, Rodrigues PB, Cantarelli VdS, Zangeronimo MG, Silva Júnior JWd, Silva LRd, et al. Energy values and chemical composition of spirulina (Spirulina platensis) evaluated with broilers. Revista Brasileira de Zootecnia. 2011;40:992-6. https://doi.org/10.1590/S1516-35982011000500008

5. Vo T-S, Ngo D-H, Kim S-K. Nutritional and pharmaceutical properties of microalgal Spirulina. Handbook of marine microalgae: Elsevier; 2015. p. 299-308. http://dx.doi.org/10.1016/B978-0-12-800776-1.00019-4

6. Kim Y-I. Folic acid supplementation and cancer risk: point. Cancer Epidemiology Biomarkers & Prevention. 2008;17(9):2220-5. https://doi.org/10.1158/1055-9965.epi-07-2557

7. Christian E, Obumneme O, Chibuzo O, Divine A, Rita N, Malachy C. Effect of a combination of ethanol extract of Ficus capensis and Cnidoscolus aconitifolius on liver and kidney function parameters of phenylhydrazine-induced anemic rats. Asian Journal of Research in Biochemistry. 2020;6(2):1-6.

8. Al Tikrity RSS, Al Rashidy AAM. Spectrophotometric determination of Folic acid (B9) by oxidative Coupling Reaction with 4-nitro-aniline. Tikrit Journal of Pure Science. 2022;27(6):28-32. http://tjps.tu.edu.iq/index.php/tjps

9. Tietz Y. Clinical Biochemistry. McGraw-Hill, New York; 2005.

10. Fossati P, Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clinical chemistry. 1982;28(10):2077-80. https://doi.org/10.1093/clinchem/28.10.2077

11. Kondrup J, Rasmussen HH, Hamberg O, Stanga Z, Group AahEW. Nutritional risk screening (NRS 2002): a new method based on an analysis of controlled clinical trials. Clinical nutrition. 2003;22(3):321-36. https://doi.org/10.1016/S0261-5614(02)00214-5

12. Tietz NW, Shuey DF. Lipase in serum--the elusive enzyme: an overview. Clinical chemistry. 1993;39(5):746-56. https://doi.org/10.1093/clinchem/39.5.746

13. Wong EC, Dietzen DJ, Bennett MJ, Haymond S. Biochemical and molecular basis of pediatric disease: Academic Press; 2021.

14. Yang JH, Choi M-H, Na C-S, Cho SS, Kim JH, Ku SK, et al. Bamboo stems (Phyllostachys nigra variety henosis) containing polyphenol mixtures activate Nrf2 and attenuate phenylhydrazine-induced oxidative stress and liver injury. Nutrients. 2019;11(1):114. https://doi.org/10.3390/nu11010114

15. Eltahan N, Elshershaby E, Diab L. Biological Studies on Quinoa and Spirulina on

Anemic Rats. Journal of Home Economics-Menofia University. 2023;33(03):17-27.
https://doi.org/10.21608/MKAS.2023.186310.1204

16. Salman M, Kamel MA, El-Nabi SEH, Ismail AHA, Ullah S, Al-Ghamdi A, et al. The regulation of HBP1, SIRT1, and SREBP-1c genes and the related microRNAs in non-alcoholic fatty liver rats: The association with the folic acid anti-steatosis. PLoS One. 2022;17(4):e0265455. https://doi.org/10.1371/journal.pone.0265455

17. ZANGENEH M, Pooyanmehr M, Zangeneh A. Evaluation of the anti-anemic potential of Glycyrrhiza glabra aqueous extract in Phenylhydrazine-treated rats. Iranian Journal of Pharmacology and Therapeutics. 2017;15(1):1-9.

18. Ahmed BS. The Protective Effects of Blue-Green Algae (Spirulina) Against Arsenic-Induced Differences in Lipid Panel and Hematological Parameters in Female Rats (Rattus norvegicus). Egyptian Journal of Veterinary Sciences. 2024;55(3):785-93.
http://dx.doi.org/10.21608/EJVS.2023.245190.1657

19. Karadeniz A, Cemek M, Simsek N. The effects of Panax ginseng and Spirulina platensis on hepatotoxicity induced by cadmium in rats. Ecotoxicology and environmental safety. 2009;72(1):231-5.
https://doi.org/10.1016/j.ecoenv.2008.02.021

20. KAMEL EA. The Protective Role of Betaine or/and Folic Acid to Reduce the Hazardous Effects Resulting from Hyperhomocysteinemia Induced in Rats.

21. Sobeh EI, Hussein S, Abdelmaksod H, Saleh HM, Hassanin WF. Therapeutic role of folic acid loaded magnetic nanoparticles against gamma-irradiation hazards in rats. Arab Journal of Nuclear Sciences and Applications. 2020;53(2):68-77. https://dx.doi.org/10.21608/ajnsa.2020.15937.1255

22. Lar S, Patel DS, Pandanaboina CS. Hepato-protective effects of blue-green alga Spirulina platensis on diclofenac-induced liver injury in rats. Malaysian journal of nutrition. 2016;22(2).

23. Al-Samarrai RRH, Al-mulisy AMH. Experimental and Acomparative Study for the effect of GlucosAmine hydrochloride as Antioxidant in Sera of Adult Male Rabbits. Tikrit Journal of Pure Science. 2016;21(2):85-97.
https://doi.org/10.25130/tjps.v21i2.976

24. Luna L. Manual of histologic staining methods of the armed forces institute of pathology. McGrow-Hill book company. 1968.

25. Presnell JK, Schreibman MP, Humason GL. Humason's animal tissue techniques. (No Title). 1997.