*Egypt. J. Vet. Sci.* **Vol. 56,** No. 1, pp. 47-60 (2025)

**Egyptian Journal of Veterinary Sciences**

**https://ejvs.journals.ekb.eg/**

# The Impact of Microalgae and Their Bioactive Compounds on Liver Well-being in Rats Subjected to Synthetic Phenolic Antioxidants

**Sara M. Omar[1], Rashed A. Alhotan[2], Elsayed O. Hussein[3], Branislav Galik[4], Nasser N. A. Zahran[5], Mahmoud Alagawany[6] and Ahmed A. Saleh[7*]**

[1] *Nutrition and Food Science Department, Faculty of Home Economics, AL-Azhar University, Egypt*
[2] *Department of Animal Production, College of Food & Agriculture Sciences, King Saud University, P. O. Box 2460, Riyadh 11451, Saudi Arabia*
[3] *AlKhumasia for Feed and Animal Products Riyadh- Olaya - Al Aqareyah 2 - Office 705 PO 8344, Riyadh 11982, Saudi Arabia*
[4] *Institute of Nutrition and Genomics, Slovak University of Agriculture in Nitra, Trieda A. Hlinku 2, 94976 Nitra, Slovakia*
[5] *Nutrition Fellow, Menoufia University Hospitals, Shebin EL-Kom, Egypt*
[6] *Poultry Department, Faculty of Agriculture, Zagazig University, Zagazig 44519, Egypt*
[7] *Department of Poultry Production, Faculty of Agriculture, Kafrelsheikh University, Kafr El-Sheikh 333516, Egypt*

**Abstract**

THIS STUDY goal was to find out if *Spirulina platensis* (SP) could help protect rats from the harmful effects of tert-butylhydroquinone (tBHQ). The animals were classified into four groups, each including seven rats. Group I consisted of healthy rats (control), while group II was subjected to an intraperitoneal administration of 100 mg/kg of tBHQ for duration of 7 days (+Ve). Group III received a dosage of 500 mg/kg (body weight) of SP and 100 mg/kg of tBHQ, while group IV was administered a mixture of 750 mg/kg of SP and 100 mg/kg of tBHQ. The rats that were given tBHQ alone had significantly higher of liver enzymes like ALT, AST, and ALP in blood. The tissue levels of the antioxidants glutathione, superoxide dismutase, total antioxidant capacity, and catalase decreased significantly in rats that were exposed to tBHQ-induced toxicity. In the experiment, rats were administered tBHQ as a positive group, leading to an increase in blood lipid profiles (such as TC, TG,) except for HDL-c as compared to the negative treatment. Administration of SP powder led to enhanced blood biochemical parameters and decreased MDA levels in rats that were injected with tBHQ. Rats exhibited an increase in their antioxidant indicators. It has been shown that there was a strong investigation we did shows a strong link between the β-actin, Bcl2, HO-1, and Nrf2 genes in the liver of the four groups that were subjected to experiments. These effects have been shown in blood lipid profile, antioxidant markers, and liver enzymes after exposure to tBHQ.

**Keywords:** tBHQ, *Spirulina platensis*, Lipid profile, Antioxidant biomarkers, Gene expression, and Liver.

## Introduction

Malnutrition is a prevalent and often disregarded issue in the poor countries of South Asia. Malnutrition significantly affects people with hepatitis. In the South Asian area, the causes of cirrhosis are different from those in the West. Hepatitis B and C remain the main causes, although the frequency of nonalcoholic fatty liver disease is growing over time [1]. Coexisting malnutrition exacerbates outcomes for people with hepatitis. Immediate focus on addressing malnutrition is necessary to enhance patient outcomes. The cause and physiological mechanisms of malnutrition in liver illnesses are complex, since a decrease in liver function impacts both macronutrients and

*Corresponding author: Ahmed Ali Saleh, E-mail: ahmed.saleh1@agr.kfs.edu.eg Tel.:00201064721165
(Received 02/01/2024, accepted 27/03/2024)
DOI: 10.21608/EJVS.2024.258700.1750

micronutrients. There is a global need for evaluating the nutritional condition of individuals with liver disease [2]. The documented incidence of malnutrition in hepatitis has significant variability, spanning from 5% to 92%. This wide range suggests the presence of either a lack of understanding or challenges in accurately detecting malnutrition, or maybe both. In addition, those with liver disease who do not have cirrhosis often have malnutrition [3]. Malnutrition might be considered a complication of cirrhosis due to its detrimental effects on the course and prognosis of the illness. Malnourished people have an increased probability of hospitalization and mortality in comparison to well-nourished ones. Furthermore, malnutrition is a substantial determinant of outcome, irrespective of other variables [4]. A protein shortage, which is characterized by insufficient protein consumption, leads to decreased levels of amino acids in the bloodstream and compromises the immune system [5]. According to the World Health Organization (WHO), about 30% of the world's population experiences malnutrition, leading to the death of 40,000 children daily owing to starvation and associated disorders, including liver degeneration in general. Malnutrition is a greater risk than the liver illness itself, since it may lead to the patient's death. Additionally, performing a liver transplant is prohibited if the patient is malnourished [6]. Antioxidants are a class of dietary additives deliberately included in nutrients to decelerate or impede oxidation events. Antioxidants include both naturally occurring and artificially produced antioxidants. Synthetic antioxidants are the most commonly used substances globally to prevent degradation and prolong the lifespan of food, pharmaceuticals, and commercial items [7]. Genes carrying antioxidant response elements (ARE) control the Keap1/Nrf-2 response, which is critically dependent on the oxidative-reduction abilities of synthetic materials. The chemical composition and redox characteristics of synthetic phenolic antioxidants play a crucial role in determining their biological effects. There is growing evidence that, in addition to the traditional mechanism, there are additional cellular targets responsible for carrying out the biological effects of conventional Nrf2 activators, including tBHQ, BHA, and other quinones [8]. When analyzing the protein content of *Spirulina*, its dry weight ranges from 65% to 71%. It is considered a non-toxic and very nutritious meal with remarkable qualities. This material is composed of 47% essential amino acids, including methionine, which is often absent in other forms of algae and cyanobacteria. Furthermore, it comprises a carbohydrate content ranging from 15% to 25%, a mineral content ranging from 8% to 13%, a fat content ranging from 3% to 7%, and a fiber content ranging from 8% to 10%. Furthermore, it contains chlorophyll, phycocyanin, carotenoids, minerals, vitamins, essential fatty acids, and several bioactive components [9]. *Spirulina* is used as a vital source containing crucial fatty acids, such as linoleic acid, linolenic acid, and phycobiliproteins, specifically emphasis on phycocyanin and allophyco-cyanin. Additionally, it contains amino acids, with leucine accounting for 10.9% of the total. Valine and isoleucine are the next most abundant amino acids. Additionally, *Spirulina* is rich in minerals such as iron. The use of *Spirulina* resulted in improvement among groups of children, adults, and those with chronic diseases that suffer from malnutrition [10]. *Spirulina* increased the protein content by 20.33% and boosted the iron content by 45%. Furthermore, the inclusion of *Spirulina* resulted in an enhancement of the overall carotenoid and polyphenol levels, as well as an increase in the antioxidant capacity of the food items. Incorporating 100 g of Spirulina-fortified food items into your diet; supplies more than 50% of the required dietary allowances (RDA) for protein, dietary fiber, iron, and zinc. Additionally, it aids in diminishing malnutrition-induced oxidative stress in the liver, spleen, and kidneys. To do this, one must decrease lipid peroxidation while simultaneously increasing glutathione and superoxide dismutase activity [11]. *Spirulina* has a substantial amount of phycocyanin, which is a prominent phytochemical and a high-protein compound. *Spirulina* consists of a total of sixteen amino acids, eight of which are essential and eight of which are non-essential, contributing to the formation of protein molecules. Hence, it may serve as a primary resource for protein malnutrition [12]. Multiple studies have shown that elevated levels of industrial antioxidants pose a significant risk as carcinogenic agents, hence increasing the potential harm to many organs, including the liver, brain, kidney, intestines, and lungs [13]. Several recent studies have shown the various pharmacological qualities of *Spirulina*, such as its ability to prevent cancer, stimulate the immune system, protect the liver from toxins, reduce inflammation, and act as an antioxidant [14]. *Spirulina platensis* shows promise in its ability to prevent and cure certain toxicities, such as tBHQ [15]. According to what we know, (tBHQ) causes hepatotoxicity, and there is little evidence to support *Spirulina's* preventive benefits. This study aims to investigate if *Spirulina platensis* may protect the liver of rats with special regard to the liver biomarkers by looking at liver functions, lipid profile, gene expression, and antioxidant.

## Experimental procedures

### Animal behavior and design methodology

Twenty-eight Sprague-Dawley white male albino rats from the Serum and Vaccine Center, Dokki, Giza; were used in the study with average weight of 150 ± 10 g. Prior to the experiment, the rats were housed together in cages that maintained a controlled temperature of 22–24 °C and a lighting schedule of

12 hours of light followed by 12 hours of darkness. This housing condition was maintained for at least 7 days. The rats were divided into four groups, each of see seven rats. Subsequently, each group was assigned a distinct food item to consume for a period of 28 days, according to the following distribution: Group I received routine (basal) diet only. Group II received diet and injected intraperitoneally with 100 mg/kg b.w tBHQ (dissolved in olive oil)/ 7days. Group III received routine (basal) diet mixed with 500 mg/kg b.w of *Spirulina platensis* powder + 100 mg/kg of tBHQ throughout the experimental period at the same time. Group IV received a mix of 750 mg/kg b.w of *Spirulina platensis* powder + 100 mg/kg of tBHQ (was purchased from Al-Gomhoria Chemical Company, Al-Bahr Street, Tanta, Egypt. The effectiveness of the procedure for tBHQ in experimental animals has been documented by previous studies [19 , 20]) at the same time. *Spirulina platensis* ready powder was purchased at the local market from Imtenan Company; Tanta, Gharbia, Egypt. The algae's carbohydrate, protein, fat, moisture, fiber, and ash contents were examined using the methodology specified in AOAC (2010). The calculation of antioxidant activity was performed using the methodologies outlined in [16]. Amino acid analysis is a technique that utilizes ion-exchange liquid chromatography. It is extensively used in several domains to accurately determine the qualitative and quantitative composition of substances. Within biochrom systems, this fundamental concept has been enhanced to provide completely automated, rapid, and very sensitive tests [17], known as classical amino acid analysis. HPLC with a Zorbax Eclipse plus C18 Agilent column that was 4.6 x 150 mm and had 5-micron particles to look at the multivitamin and mineral content in *Spirulina* was used. The mobile phase included a mixture of methanol and air in a proportion of 35:65. A flow rate of 1.5 ml/minute was established, and a UV detector operating at a wavelength of 550 nm was used. The volume of the injection was 20 ml. To determine the retention time, a process is followed where one gram of *Spirulina* biomass is extracted using 100 mL of water and then transferred to the injection column. Consequently, the duration for which each vitamin and mineral standard is kept was adjusted based on the method used. Utilizing the specified procedure, HPLC was used to detect and quantify the phenolic constituents in *Spirulina platensis* [18]. Qualitative analysis showed that the *Spirulina platensis* powder (Oven-dried), contained all flavonols, except caffeic acid and quercetin (Tables 1, 2 & 3, and Figu. 1).

**Biochemical parameters**

Upon completion of the experiment, anesthesia was administered to all rats using R550 is a Multi-output Laboratory Small Animal Anesthesia Machine, which is suitable for the simultaneous anesthesia of 1-5 rats, mice, cats, rabbits, and other animals. Each channel for anesthesia can be controlled independently. The gas flow of the induction box can be adjusted independently, with a range of 0-2.0L/min, and blood samples were obtained from the hepatic portal vein to assess the biochemical variables. The blood samples were placed into tubes containing heparin in order to analyze the biochemical characteristics. The samples were there-after subjected to centrifugation at a speed of 3,000 revolutions per minute for duration of 15 minutes in order to isolate the serum. The levels of serum total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-c) were measured using enzymatic colorimetric techniques [21]. The VLDL-c concentration, measured in milligrams per deciliter, is calculated by dividing the triglyceride level by 5 [22]. The LDL-c was calculated using the following equation: The formula for calculating LDL cholesterol (LDL-c) in milligrams per deciliter (mg/dL) is as follows: LDL-c = Total cholesterol (TC) – [HDL cholesterol (HDL-c) + Very low-density lipoprotein cholesterol (VLDL-c)] [23]. The quantification of AST, ALT, AST/ALT ratio, and ALP was performed using the methods outlined in reference [24]. The liver was extracted, and its tissues were washed in a chilled 0.9% saline solution (by weight or volume), then quantified and stored at a temperature of -70°C. We checked the levels of malondialdehyde (MDA) and the activities of superoxide dismutase (SOD), glutathione (GSH), and catalase (CAT), following the steps described in reference [25]. The measurement of total antioxidant capacity (TAC) was also conducted [26].

**Liver histopathology**

The liver was preserved using a 10% solution of neutral buffered formalin immediately after removing it from the animals. The fixed tissues were thereafter subjected to known histological tests [27].

**Gene expression**

RNA extraction was performed using rat liver. The RNA globules were dissolved using diethylpyro-carbonate (DEPC) water. The RNA concentration was measured using spectrophotometry at an optical density (OD) ratio of 260/280 [28]. The semi-quantitative reverse transcription PCR used 3 µg of RNA. This process included subjecting the plate to denaturation in PCR thermocycler (Bio-Rad T100TM) at a temperature of 70 degrees Celsius for a period of 5 minutes. In addition, 0.5 nanograms of oligo dT primers were used. Two microliters of 10X RT buffer, two microliters of 10 mM dNTP, and one microliter of 100 M reverse transcriptase were mixed to make cDNA. The mixture was incubated at 42 ºC for 1 hour and then heated at 70 ºC for 10 minutes to assure deactivation of the enzyme. To evaluate the gene expression levels were quantified using

densitometry, with the mRNA expression of β-actin serving as a reference standard. The 2-ΔΔCT method was used to measure the expression levels of these genes using real-time PCR. The endogenous reference gene, actin, was used to standardize the analyzed genes. The CT values were used to examine the alterations in gene density and mRNA expression using a comparative approach [29].

**Statistical analysis**

The data were analyzed using the SPSS program (Version 17.0). The data were reported as the mean±SE. Following a one-way ANOVA, Duncan's multiple range tests compared the data. The statistical significance criterion was set at the $p \leq 0.05$ level.

**Findings and analysis**

The chemical makeup of *Spirulina platensis* is shown in Table 1 per 100 g on a dry weight basis, including its protein, carbohydrate, ash, lipids, moisture, and fibers (64.4±1.43, 15.50±0.40, 12.05±0.21, 9.23±0.26, 6.50±0.06 and 8.25±0.25 (g/100 g DW), respectively. *Spirulina* also has a significant level of total antioxidant activity, measuring at 42%. The statistics presented here align with [30 , 31], which indicate that *Spirulina* has a substantial protein content. The protein composition was of high quality since it included both required and nonessential amino acids. Long-term malnutrition causes “anemia,” which affects the general population of developing countries, as well as many other diseases such as goiter, hypokalemia, tooth decay, and vitamin deficiency, complications of liver disease, which have existed for many years [32]. Countless well-organized intervention efforts failed to make any improvement whatsoever. Considering the inclusion of *Spirulina* as a nutritional supplement to enhance the food product with protein, calcium, iron, vitamin B12, phosphorus, and multivitamins included in the nutritional assessment of these people may be ascorbic acid, vitamins B, D, and E, as well as minerals [14]. *Spirulina* is recognized for its many medicinal uses, encompassing antigenotoxic, anti-inflammatory, antidiabetic, antioxidant, anti-cancer, hepatoprotective, and neuroprotective properties, which are attributed to the presence of phytochemicals [9] (Table 3). The current results showed that when comparing the treated group with the control regarding liver enzymes, it was noted that tBHQ led to a significant increase in these enzymes. And when comparing the treatment groups of *Spirulina* powder with its different concentrations, a significant decrease in liver enzymes was observed, but group with the highest concentration of *Spirulina* powder, which was 750 mg, was the best group (Table 4). Tert-butylhydroquinone may have caused the damage seen in the positive group by creating certain lesions that change how permeable liver cell membranes are. This phenomenon takes place when the metabolic byproduct "glycidamide" binds with the functional groups of membrane proteins [33]. Nevertheless, the levels of these liver enzymes exhibited a reduction that was directly proportional to the dosage after administration of *Spirulina* powder. A previous study found that *Spirulina* powder has a hepatoprotective effect against high concentrations of tBHQ-induced acute hepatotoxicity in rats. It does this by lowering lipid peroxidation, which strengthens the hepatocyte membrane, boosts the activity of antioxidant enzymes, and lowers the inflammatory response, and shows radical scavenging ability. The presence of abundant phycocyanin and phenolic chemicals, together with the antioxidant activity of *Spirulina* powder, might explain these data [34]. As for the lipid profiles, and upon comparing the injured group with the negative group, a notable and statistically significant rise in the proportions of variables was observed except for HDL-c, and this indicates the extent of the effect of tBHQ on those variables. The fourth group, which was fed 750 mg of *Spirulina* powder, was the best group when comparing different concentrations of *Spirulina* powder with the control group (Table 5). *Spirulina* powder reduces lipid levels by lowering cholesterol and triglyceride levels in the blood, as it increases HDL-c levels, reduces LDL-c and VLDL-c [35]. Results were acceptable with [36]. *Spirulina* is believed to enhance the blood lipid profile and reduce oxidative stress. *Spirulina* considered as a good supplementation for patients with liver and other chronic diseases. *Spirulina* powder has the potential to decrease the production of fats inside the body. The pentose phosphate route may cause less glucose-6-phosphatase activity, which could lower the ratio of glutathione to oxidized glutathione. This could cause NADPH to change into $NADP^+$. *Spirulina* powder may have a significant role in the generation of abundant $NADP^+$, which controls the synthesis of fats and mitigates tissue damage caused by oxidative stress [37]. *Spirulina* powder has therapeutic properties, including the prevention and reduction of damages caused by hyperlipidemia, as well as antioxidant activity [38]. Research conducted on several animal models has shown that *Spirulina* effectively lowers plasma and hepatic levels of TC, LDL-c, and TG. Similarly, research involving human has also indicated a substantial reduction in TC, LDL-c, and TG levels. Additional research has shown that *Spirulina* may reduce TG levels by affecting lipoprotein metabolism [39]. Free radicals are associated with several metabolic processes, such as glucose autoxidation, the polyol pathway, and protein glycation. Lipid peroxidation may result in harm to proteins, lipids, carbohydrates, and nucleic acids. Additionally, it may serve as a catalyst for

tissue damage in cardiovascular illnesses and the degradation of cell membranes and internal cellular components [40]. Several chemicals have the potential to disrupt the normal functioning and metabolism of the liver. Oxidative stress is a significant contributor to liver damage [41]. Excessive amounts of tBHQ, a chemical compound, may lead to oxidative stress and harm hepatocytes [42]. Table 6 displays the impact of *Spirulina* algae as a substitute for casein, a low-cost animal protein, with equivalent biological value on GSH, SOD, CAT, and TAC. Many more antioxidant enzymes (GSH, SOD, CAT, and TAC) were found in liver tissue from *Spirulina* powder groups (III & IV) unlike the group that had unfavorable traits. On the contrary, the MDA exhibited a significant increase in the positive group in contrast to the groups subjected to treat with *Spirulina* powder (III & IV). By seeing that antioxidant enzymes (GSH, SOD, CAT, and TAC) were working less well and the level of MDA going up, the study proved that oxidative stress was present. The administration of *Spirulina* powder led to an augmentation in antioxidant enzymes, which is associated with the antioxidant characteristics of *Spirulina* powder [43]. The data suggest that *Spirulina* powder exhibits antioxidant properties attributed to its different antioxidant components [44]. Also, giving *Spirulina* powder increased the activity of antioxidant enzymes and the amount of GSH in the liver and kidney tissues by a large amount [45]. Research examining the distribution of total phenolic compounds in commercial goods has shown differences. However, it has been reported that *Spirulina* typically includes chlorogenic acid, synaptic acid, salicylic acid, trans-cinnamic acid, and caffeic acid [7]. *Spirulina* contains antioxidant compounds, including phycobilins and phycocyanins, which may inhibit the function of catalytic enzymes like lipoxygenase and cyclooxygenase or enhance the activity of enzymes like GPX, CAT, and SOD [34]. The polyphenols have been shown to have anti-inflammatory, antiviral, antioxidant, antithrombotic, vasodilatory, antidiabetic, neuroprotective, hepatoprotective, and anti-carcinogenic properties [46]. Gene expression was conducted on four groups using the genes Nrf2, HO-1, BCl-2, and β-actin are represented by the data shown in Table 7 and Figure 2. During our analysis, we discovered a substantial association between the β-actin gene and the comparison groups; control negative versus tBHQ, 500 mg/kg *Spirulina* powder + 100 mg/kg tBHQ vs. (+Ve), and 750 mg/kg *Spirulina* + 100 mg/kg tBHQ versus (+Ve) ($p \leq 0.05$). A significant correlation exists between administering 500 mg/kg of Spirulina powder combined with 100 mg/kg of tBHQ and administering 750 mg/kg of *Spirulina* powder combined with 100 mg/kg of tBHQ, as compared to the control group ($p \leq 0.05$) for both genes. Nrf2 gene was compared in three different conditions: control against positive, positive versus a combination of 500 mg/kg *Spirulina* powder and 100 mg/kg tBHQ, and positive versus a combination of 750 mg/kg *Spirulina* powder and 100 mg/kg tBHQ. The statistical significance for all comparisons was $p \leq 0.05$. Figure 3 illustrates the prevalence of 4 groups among the genes analyzed in our research. Documented reports have shown a related between genotoxicity and certain synthetic antioxidants, such as tBHQ. tBHQ has also been associated with genotoxicity and cytotoxicity in experimental animal studies. *In vitro* experiments have shown that human peripheral blood cells exhibit genotoxic effects when exposed to tBHQ. This genotoxicity has also been seen in previous investigations [47]. Various investigations have examined the genotoxic effects of tBHQ both in laboratory settings and in living organisms using various genetic indicators [44]. A favorable outcome was reported in a forward mutation experiment conducted on mouse lymphoma cells at the thymidine kinase (TK) gene, but only when rat liver S9 was present. Several investigations have shown that tBHQ causes structural chromosomal abnormalities in laboratory settings without the addition of S9 and/or with the addition of S9 [45]. Previous researchers have studied the genotoxic mechanism of tBHQ in V79 cells [7]. Within this cellular framework, tBHQ triggered the formation of micronuclei that exhibited both CREST $^{(-)}$ characteristics, signifying chromosomal breakage, and CREST $^{(+)}$ characteristics, suggesting chromosome loss. CAT prevented the development of CREST $^{(-)}$ micronuclei, whereas hypoxanthine/xanthine oxidase promoted their creation. GSH effectively prevented the development of both CREST $^{(+)}$ and CREST $^{(-)}$ micronuclei [48]. Rats with higher liver LPO levels show that ROS and oxidative stress may be involved in the cell death and DNA damage caused by tBHQ. This study discovered that genotype had no substantial impact when examining the genes by RT-PCR.

**Histopathological examination**

Photo 1 displays a portion of healthy liver control group. It shows a central vein (shown by red arrows) and portal tracts (indicated by black arrows), which consist of a portal venule, a portal arteriole, and bile ducts. Hepatocyte cords of normal size surround these structures (blue arrows indicate this). The image was magnified 100 times and dyed with hematoxylin and eosin (H&E×100). Photo 2 depicts a segment of a healthy liver from the control group, serving as negative reference. Red arrow indicates the central vein, and a black arrow denotes the portal tracts, which include a portal venule, portal arteriole, and bile ducts. Hepatocyte cords of normal size

surround these structures (blue arrows indicate this). The image was magnified 100 times and dyed with hematoxylin and eosin (H&E×100). Photo 3 point to section of the liver group exposed to alcohol (+Ve) displayed significant dilation and congestion (shown by red arrows), accompanied by a high number of chronic inflammatory cells (indicated by the black arrow) and regions of degraded hepatocytes (indicated by the blue arrows) and dyed with hematoxylin and eosin (H&E×100). The liver area in Photo 4 shows how tBHQ poisoning has affected that area (+Ve) displayed a concentrated area of cell death (necrosis) that merged together (focal confluent necrosis) (blue arrow). Additionally, there was an artery in the portal area that was filled with blood (congested portal arteriole) (red arrow), and it was surrounded by numerous cells associated with long-term inflammation (chronic inflammatory cells) (black arrows) (H&E X100). Photo 5 depicts a segment of the liver from the group that received a dosage of 500 mg/kg of *Spirulina platensis* powder and 100 mg/kg of tBHQ. It displays a moderate accumulation of chronic inflammatory cells surrounding a slightly enlarged and congested portal venule (black arrows indicate this). Additionally, there are degenerated hepatocytes surrounding the inflammatory cells (indicated by blue arrows). The image was magnified 100 times and stained with (H&E ×100). Photo 6 explains a portion of liver sample from the treated group, the groups were administered a dose of 500 mg/kg of *Spirulina platensis* powder and 100 mg/kg of tBHQ. The image shows a slight enlargement and congestion of the portal venule and central vein (indicated by red arrows), as well as a mild chronic inflammation in the adjacent lobe (indicated by a black arrow). Additionally, there are degenerated hepatocytes present (indicated by blue arrows). The image was captured using an H&E staining technique at a magnification of 100X. Photo 7 depicts a specific section of the liver group that had treatment with a dosage of 750 mg/kg of *Spirulina platensis* powder and 100 mg/kg of tBHQ. The red arrow in the image indicates a moderately dilated and congested portal venule. Surrounding the venule is a mild infiltration of chronic inflammatory cells, as indicated by the black arrow. The area also contains normal hepatocytes without any signs of degeneration, as indicated by the blue arrows. The image was magnified 100 times and stained with H&E. Photo 8 displays a portion of liver sample from the treated group, which received a dosage of 750 mg/kg of *Spirulina platensis* powder and 100 mg/kg of tBHQ. The image shows a central vein and portal venule of normal size, as well as a slight infiltration of chronic inflammatory cells (indicated by the black arrows). The blue arrows indicate that the surrounding hepatocytes appear normal and do not exhibit any signs of degeneration. The image was captured using an H&E staining technique at a magnification of 100x.

## Conclusion

The primary contributing factor to this is the composition of the used algae as well as their capacity to produce a diverse array of chemical compounds that are of significance in both biological and commercial contexts. *Spirulina platensis* is widely acknowledged for its high nutritional value. *Spirulina platensis* exhibits antioxidant and anti-inflammatory effects, making it potentially beneficial for individuals with chronic diseases and malnutrition, particularly those experiencing hepatotoxicity due to elevated levels of (tBHQ). Cost-effective food processing techniques can generate a sizable amount of biomass, ensuring the acquisition of natural components with significant nutritional value. Several naturally occurring substances have the potential to be used in the production of functional foods and various bioactive compounds, including antioxidants. Consequently, a growing cohort of academics and experts worldwide are advocating for heightened cultivation of *Spirulina*.

*Author contributions*

All authors have thoroughly reviewed and consented to the final version of the work that has been made publicly available.

*Funding statement*

The authors would like to acknowledge the Researchers Supporting Project number (RSPD2024R581) for their kind financial assistance.

*Informed consent statement***:** N/A.

*Conflicts of interest*

The authors assert that they have no conflicting interests.

*Acknowledgments*

The authors wish to express their gratitude to King Saud University in Riyadh, Saudi Arabia, for providing financial support under the Researchers Supporting Project number (RSPD2024R581).

**TABLE 1. Presents the chemical composition of *Spirulina platensis*, measured in grams per 100 grams of dry weight**

| Composition of *Spirulina platensis* | Approximate (%) |
|---|---|
| **Carbohydrates** | 15.50±0.40 |
| **Protein** | 64.4±1.43 |
| **Ash** | 12.05±0.21 |
| **Lipids** | 9.23±0.26 |
| **Fibers** | 8.25±0.25 |
| **Moisture** | 6.50±0.06 |
| **Total antioxidant activity** | 42 % |
| **Amino acids (g/100 g)** | |
| **Threonine** | 3.97 |
| **Tryptophan** | 0.959 |
| **Isoleucine** | 3.259 |
| **Lysine** | 4.025 |
| **Leucine** | 5.947 |
| **Methionine** | 1.156 |
| **Phenylalanine** | 3.777 |
| **Cystine** | 0.762 |
| **Tyrosine** | 2.596 |
| **Valine** | 3.312 |
| **Histidine** | 1.085 |
| **Arginine** | 3.186 |
| **Alanine** | 4.515 |
| **Aspartic acid** | 4.793 |
| **Glutamic acid** | 6.386 |
| **Proline** | 3.382 |
| **Glycine** | 3.125 |
| **Serine** | 2.798 |

**TABLE 2. Presents the content of vitamins and minerals in *Spirulina platensis*, measured in grams per 100 grams of dry weight**

| Minerals | mg 100 $g^{-1}$ | Vitamins | mg 100 $g^{-1}$ |
|---|---|---|---|
| **Chromium** | 0.32 | **Riboflavin B2** | 5 |
| **Calcium** | 800 | **Vitamin B12** | 0.36 |
| **Copper** | 1.5 | **Vitamin E** | 150 α-tocopherol eq |
| **Magnesium** | 500 | **Thiamin B1** | 3.7 |
| **Iron** | 170 | **Provitamin A** | 2.530.000 IU $kg^{-1}$ |
| **Manganese** | 6 | **Vitamin K** | 2.8 |
| **Potassium** | 1500 | **Vitamin B6** | 0.9 |
| **Phosphorus** | 900 | **(β-carotene)** | 160 |
| **Zinc** | 4 | **Folic acid** | 0.02 |
| **Sodium** | 800 | **Pantothenic acid** | 0.2 |
| | | **Biotin** | 0.01 |
| | | **Niacin B3** | 16 |

**TABLE 3. Presents the phenolic chemicals found in *Spirulina platensis* powder**

| Compounds | Rt | *Spirulina* powder |
|---|---|---|
| **Catechin** | 11.48 | + |
| **Gallic acid** | 6.21 | + |
| **Caffeic acid** | 13.25 | - |
| ***p*-Cumaric acid** | 18.79 | + |
| ***p*-Hydroxybenzoic acid** | 14.17 | + |
| **Ferulic acid** | 18.83 | + |
| **Quercetin** | 29.29 | - |
| **Kaempferol** | 36.97 | + |
| **Genistein** | 34.97 | + |

**Note:** Rt. (retention time) indicates the time it takes for a compound to go through a chromatography column. (+) signifies that a compound has been identified, while (-) indicates that a compound has not been found.

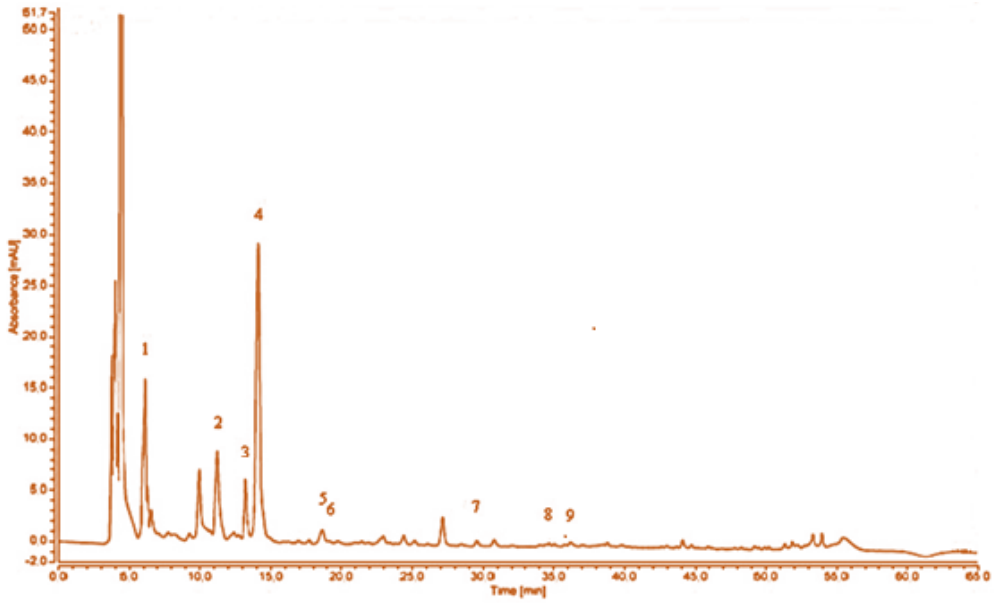



**Fig. 1. Displays a chromatography graph showing the phenolic components of Spirulina platensis powder. The graph measures the absorbance at a wavelength of 272 nm. The determination of peaks was conducted by comparing the retention time and UV-vis spectra with commercially available reference compounds. The peaks observed in the analysis are as follows: gallic acid (1), catechin (2), caffeic acid (3), p-hydroxy-benzoic acid (4), p-coumaric acid (5), ferulic acid (6), quercetin (7), genistein (8), and kaempferol (9).**

**TABLE 4. impact of *Spirulina platensis* powder on hepatic enzymes in rats with tBHQ-induced toxicity**

| Groups | AST (U/L) | ALT (U/L) | AST/ALT ratio | ALP (U/L) |
|---|---|---|---|---|
| **GI (-Ve)** | 102.30±1.22$^{d}$ | 27.27±1.19$^{c}$ | 3.76±0.12$^{c}$ | 115.20±1.22$^{d}$ |
| **GII (+Ve)** | 290.35±1.10$^{a}$ | 57.50±1.07$^{a}$ | 5.05±0.08$^{a}$ | 189.33±1.45$^{a}$ |
| **GIII** | 210.33±1.18$^{b}$ | 41.37±2.18$^{b}$ | 5.11±0.23$^{a}$ | 135.37±1.56$^{b}$ |
| **GIV** | 119.67±1.25$^{c}$ | 26.83±1.32$^{c}$ | 4.48±0.18$^{b}$ | 124.53±1.32$^{c}$ |

The results are presented as the Mean±SE. Values that have distinct letters in each row exhibit a significant difference ($P \leq 0.05$), but the difference between values that share some or all of the same letters is not statistically significant. tBHQ refers to tert-butylhydroquinone, SP stands for Spirulina platensis powder, AST is an abbreviation for aspartate aminotransferase, ALT stands for Alanine aminotransferase, and ALP represents alkaline phosphatase.

**TABLE 5. impact of *Spirulina platensis* powder on lipid profile in rats with tBHQ-induced toxicity**

| Groups | TC (mg/dl) | TG (mg/dl) | HDL-c (mg/dl) | LDL-c (mg/dl) | VLDL-c (mg/dl) |
|---|---|---|---|---|---|
| **GI (-Ve)** | 86.23±1.21$^{d}$ | 77.33±1.24$^{d}$ | 53.43±1.27$^{a}$ | 17.60±0.34$^{d}$ | 15.47±0.25$^{d}$ |
| **GII (+Ve)** | 283.43±1.78$^{a}$ | 192.73±1.53$^{a}$ | 41.47±1.28$^{c}$ | 202.71±2.87$^{a}$ | 39.25±0.45$^{a}$ |
| **GIII** | 168.83±2.05$^{b}$ | 151.73±1.53$^{b}$ | 47.37±1.24$^{b}$ | 90.97±0.77$^{b}$ | 30.51±0.17$^{b}$ |
| **GIV** | 132.57±1.60$^{c}$ | 110.07±1.16$^{c}$ | 49.73±1.35$^{a}$ | 60.82±2.69$^{c}$ | 22.01±0.23$^{c}$ |

The results are presented as the Mean±SE. Values that have distinct letters in each row exhibit a significant difference (P≤0.05), but the difference between values that share some or all of the same letters is not significant. tBHQ stands for tert-butylhydroquinone, SP refers to Spirulina platensis powder, TC represents total cholesterol, TG stands for triglycerides, HDL-c refers to high-density lipoprotein cholesterol, LDL-c represents low-density lipoprotein cholesterol, and VLDL-c stands for very low-density lipoprotein cholesterol.

**TABLE 6. impact of *Spirulina platensis* powder on antioxidants indicators in rats with tBHQ-induced toxicity**

| Groups | GSH (nMol ) | SOD (nMol ) | CAT (nMol) | TAC (nMol) | MDA (nMol) |
|---|---|---|---|---|---|
| **GI (-Ve)** | 1.95±0.02$^{a}$ | 42.38±1.18$^{a}$ | 1.01±0.02$^{a}$ | 1.40±0.03$^{a}$ | 3.70±0.02$^{d}$ |
| **GII (+Ve)** | 0.71±0.01$^{d}$ | 18.70±0.01$^{d}$ | 0.43±0.01$^{d}$ | 0.38±0.01$^{d}$ | 15.61±0.03$^{a}$ |
| **GIII** | 1.30±0.03$^{c}$ | 29.30± 0.02$^{c}$ | 0.60±0.03$^{c}$ | 0.72±0.02$^{c}$ | 9.05±0.04$^{b}$ |
| **GIV** | 1.58±0.02$^{b}$ | 37.15±0.03$^{b}$ | 0.89±0.02$^{b}$ | 1.08±0.01$^{b}$ | 5.70±0.01$^{c}$ |

The results are presented as the Mean±SE. Values that have distinct letters in each row exhibit a significant difference (P≤0.05); however, the disparity between values that share either partially or entirely the same letters are not statistically significant. tBHQ refers to tert-butylhydroquinone, SP stands for Spirulina platensis powder, GSH represents glutathione, SOD denotes superoxide dismutase, CAT stands for catalase, TAC refers to total antioxidant capacity, and MDA represents malondialdehyde.

**TABLE 7. the primer sequence used for quantitative real-time PCR in liver rats with tBHQ-induced toxicity**

| Gene | Entry number | Product size (bp) | Primer direction | Sequence |
|---|---|---|---|---|
| **β-actin** | NM_007393.4 | 140 bp | + | CCAGCCTTCCTTCTTGGGTA |
| | | | - | CAATGCCTGGGTACATGGTG |
| **Bcl2** | NM_009740 | 154 bp | + | AGCCTGAGAGCAACCCAAT |
| | | | - | AGCGACGAGAGAAGTCATCC |
| **HO-1** | NM_010442.1 | 125 bp | + | CGCCTCCAGAGTTTCCGCAT |
| | | | - | GACGCTCCATCACCGGACTG |
| **Nrf2** | NM_010902.3 | 139 bp | + | CGCCTGGGTTCAGTGACTCG |
| | | | - | AGCACTGTGCCCTTGAGCTG |

**Note:** (+) indicates sense, and (-) indicates antisense. HO-1 refers to heme oxygenase-1, β-actin is the term for beta-actin, BCL2 stands for B-Cell leukemia/lymphoma 2, and Nrf2 symbolizes nuclear-related factor-2.

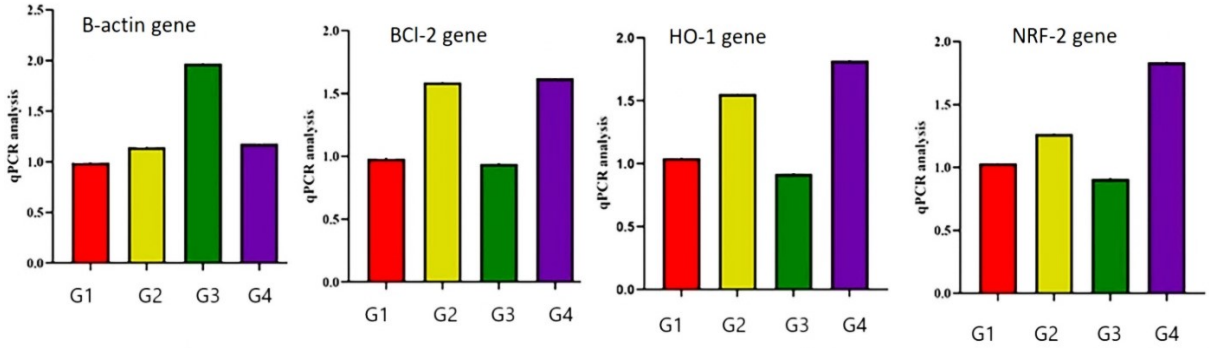
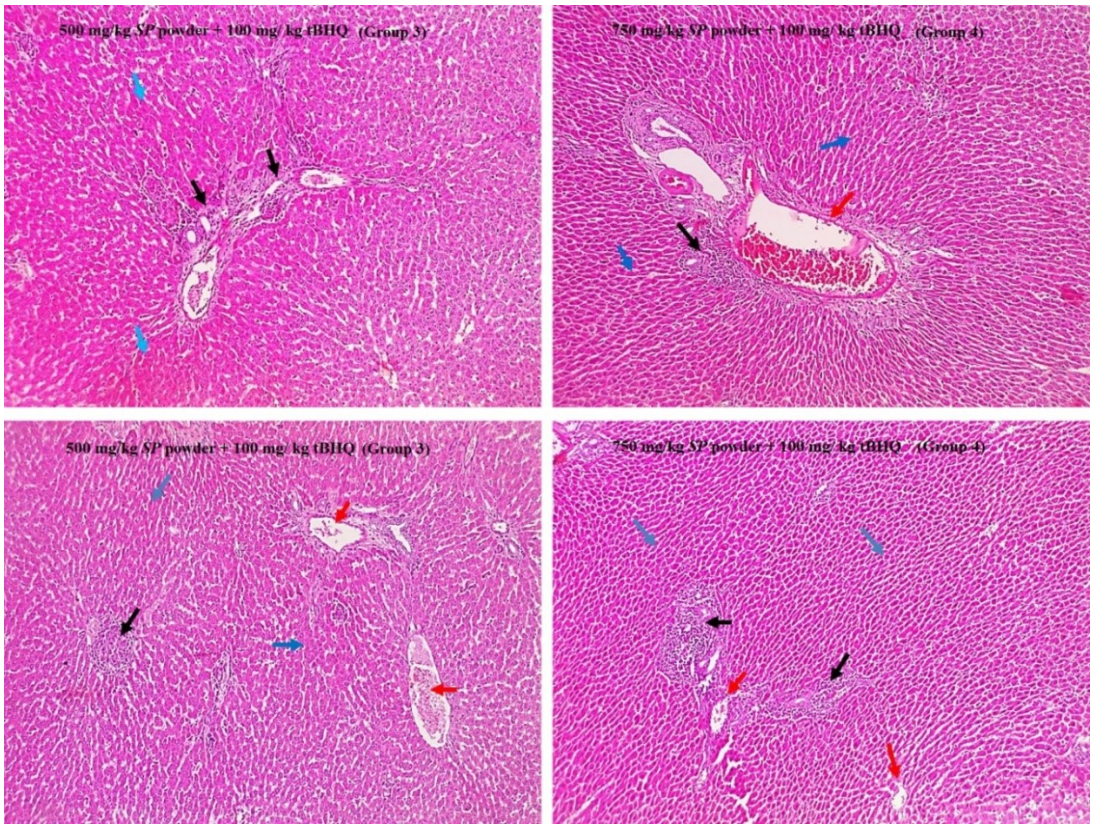



**Fig. 2. ANOVA analysis point to the significant correlation between β-actin, Bcl2, HO-1, and Nrf2 genes in 4 groups of rats induced by tBHQ toxicity.**




**Photos A1 and A2. show histological sections of the liver stained with hematoxylin and eosin (H×E). It was clear that the liver from the negative control group had a central vein (shown by red arrows) and portal tracts (shown by black arrows, which stand for a portal venule, a portal arteriole, and bile ducts). The hepatocytes were organized in regular-sized cords, as indicated by blue arrows. The liver sections B1 and B2 of the group that consumed tBHQ (called the "positive control") showed clear signs of swelling and expansion (shown by the red arrows), as well as a lot of chronic inflammatory cells (shown by the black arrows) and areas of damaged hepatocytes (shown by the blue arrows). The liver sections C1 and C2 from the group that received 500 mg/kg SP powder and 100 mg/kg tBHQ displayed a moderate infiltration of chronic inflammatory cells (indicated by black arrows) and hepatocytes that were degrading (indicated by blue arrows), as well as a slightly enlarged and swollen portal venule. The moderately dilated and congested portal venule in the liver sections of the D1 and D2 groups, which received 750 mg/kg SP powder and 100 mg/kg tBHQ, respectively, was visible (red arrow). A modest infiltrate of chronic inflammatory cells was present around this venule (the black arrow indicates this). The blue arrows, which represent the adjacent hepatocytes, exhibited no evidence of degeneration.**

**References**


1. Siddiqui, A.T.S., Parkash, O. and Hashmi, S.A. Malnutrition and liver disease in a developing country. *World J. Gastroenterol.,* **27**(30), 4985-4998 (2021). doi: 10.3748/wjg.v27.i30.4985
2. Pimpin, L. Hepahelth Project Report. Europeal Association for the Study of the Liver: Geneva, Switzerland (2018).
3. Bruch, J.P., Álvares-DA-Silva, M.R., Alves, B.C. and Dall'Alba V. Reduced hand grip strength in overweight and obese chronic hepatitis C patients. *Arquivos de Gastroenterologia,* **53**, 31-35 (2016). doi:10.1590/S00042803201600010007
4. Ribeiro, H. S., Maurício, S. F., da Silva, T. A., de Vasconcelos Generoso, S., Lima, A. S. and Correia, M. I. T. D., Combined nutritional assessment methods to predict clinical outcomes in patients on the waiting list for liver transplantation. *Nutrition,* **47**, 21-26 (2018). doi:10.1016/j.nut.2017.09.014
5. Chang, K. V., Chen, J. D., Wu, W. T., Huang, K. C., Lin, H. Y. and Han, D. S. Is sarcopenia associated with hepatic encephalopathy in liver cirrhosis? A systematic review and meta-analysis. *Journal of the Formosan Medical Association,* **118**(4),833-842(2019) doi:10.1016/j.jfma.2018.09.011
6. Furuya, J., Suzuki, H., Hidaka, R., Akatsuka, A., Nakagawa, K., Yoshimi, K. and Minakuchi, S., Oral health status and its association with nutritional support in malnourished patients hospitalised in acute care. *Gerodontology,* **39** (3), 282-290(2022), doi:10.1111/ger.12582
7. Khezerlou, A., pouya Akhlaghi, A., Alizadeh, A. M., Dehghan, P. and Maleki, P. Alarming impact of the excessive use of tert-butylhydroquinone in food products: A narrative review. *Toxicology Reports,* **9**, 1066-1075 (2022). doi:10.1016/j.toxrep.2022.04.027
8. Schmidlin, C.J. The Temporal and Contextual Relationship of NRF2 Activation and Cancer. The University of Arizona. (2021).
9. Abu-Taweel, G. M., Antonisamy, P., Arokiyaraj, S., Kim, H. J., Kim, S. J., Park, K. H. and Kim, Y. O. Spirulina consumption effectively reduces anti-inflammatory and pain related infectious diseases. *Journal of Infection and Public Health,* **12**(6),777-782(2029), doi:10.1016/j.jiph.2019.04.014
10. Okechukwu, P. N., Ekeuku, S. O., Sharma, M., Nee, C. P., Chan, H. K., Mohamed, N. and Froemming, G. R. A. In vivo and in vitro antidiabetic and antioxidant activity of spirulina. *Pharmacognosy Magazine,* **15**(Suppl 1), S17-S29(2019). GALE|A584164914
11. Kumar, R., Sharma, V., Das, S., Patial, V. and Srivatsan, V. Arthrospira platensis (Spirulina) fortified functional foods ameliorate iron and protein malnutrition by improving growth and modulating oxidative stress and gut microbiota in rats. *Food & Function,* **14**(2), 1160-1178 (2023). doi:10.1039/D2FO02226E
12. Anvar, A. and Nowruzi, B. Bioactive properties of spirulina: A review. *Microb. Bioact.,* **4**, 134-142, (2021). doi:10.25163/microbbioacts.412117B0719110521
13. Felter, S.P., Zhang, X. and Thompson, C. Butylated hydroxyanisole: Carcinogenic food additive to be avoided or harmless antioxidant important to protect food supply?. *Regulatory Toxicology and Pharmacology,* **121**, 104887, (2021). doi:10.1016/j.yrtph.2021.104887
14. Kanojia, S. Development of value-added cookies incorporated with Spirulina platensis for the mitigation of malnutrition in children. *Agriculture,* **5** (1), 102-108 (2022).
15. Natalia, E., Nadezhda, M., Ekaterina, Z., Varvara, M. and Ekaterina, L. Ultrasonic and microwave activation of raspberry extract: antioxidant and anti-carcinogenic properties. *Foods and Raw Materials,* **7**(2), 264-273(2019) doi: 10.21603/2308-4057-2019-2-264-273
16. Moukette, B., Constant Anatole, P., NyaBiapa, C.P., Njimou, J.R. and Ngogang, J.Y. Free radicals quenching potential, protective properties against oxidative mediated ion toxicity and HPLC phenolic profile of a Cameroonian spice: Piper guineensis. *Toxicology Reports,* **2**,792-805 (2015) . doi:10.1016/j.toxrep.2015.02.007
17. Pearson, D. The Chemical Analysis of Food. 6th. London: Church Hill, Livingstone, (1981).
18. Liestianty, D., Rodianawati, I., Arfah, R. A., Assa, A., Patimah Sundari and Muliadi. Nutritional analysis of spirulina sp to promote as superfood candidate. in IOP Conference Series: *Materials Science and Engineering. IOP Publishing,***509**, 012031(2019), doi 10.1088/1757-899X/509/1/012031
19. Jin, W., Ni, H., Dai, Y., Wang, H., Lu, T., Wu, J. and Liang, W. Effects of tert-butylhydroquinone on intestinal inflammatory response and apoptosis following traumatic brain injury in mice. *Mediators of Inflammation,* **2010 ,** ID 502564 (2010). doi:10.1155/2010/502564
20. Zhang, Y., Fang Liu, F., Bi, X., Wang, S., Wu, X. and Jiang, F. The antioxidant compound tert-butylhydroquinone activates Akt in myocardium, suppresses apoptosis and ameliorates pressure overload-induced cardiac dysfunction. *Scientific Reports,* **5**(1), 13005, (2015) .doi: 10.1038/srep13005

21. Chu, S.-Y., Jung, J.-H., Park, M.-J. and Kim, S.-H. Risk assessment of metabolic syndrome in adolescents using the triglyceride/high-density lipoprotein cholesterol ratio and the total cholesterol/high-density lipoprotein cholesterol ratio. *Annals of Pediatric Endocrinology & Metabolism,* **24**(1), 41 (2019) .doi: 10.6065/apem.2019.24.1.41

22. Bijland, S., Pieterman, E. J., Maas, A. C., van der Hoorn, J. W., van Erk, M. J., van Klinken, J. B. and Rensen, P. C. Fenofibrate increases very low density lipoprotein triglyceride production despite reducing plasma triglyceride levels in APOE* 3-Leiden. CETP mice. *Journal of Biological Chemistry,* **285**(33), 25168-25175(2010). doi:10.1074/jbc.M110.123992

23. Sampson, M., Ling, C., Sun, Q., Harb, R., Ashmaig, M., Warnick, R. and Remaley, A. T. A new equation for calculation of low-density lipoprotein cholesterol in patients with normolipidemia and/or hypertriglyceridemia. *JAMA Cardiology,* **5**(5), 540-548 (2020). doi:10.1001/jamacardio.2020.0013

24. Cheng, C. H., Chu, C. Y., Chen, H. L., Lin, I. T., Wu, C. H., Lee, Y. K. and Bair, M. J. Subgroup analysis of the predictive ability of aspartate aminotransferase to platelet ratio index (APRI) and fibrosis-4 (FIB-4) for assessing hepatic fibrosis among patients with chronic hepatitis C. *Journal of Microbiology, Immunology and Infection,* **53**(4), 542-549 (2020). doi:10.1016/j.jmii.2019.09.002

25. Alatawi, F.S., Faridi,U.A. and Alatawi,M.S. Effect of treatment with vitamin D plus calcium on oxidative stress in streptozotocin-induced diabetic rats. *Saudi Pharmaceutical Journal,* **26**(8), 1208-1213 (2018) .doi:10.1016/j.jsps.2018.07.012

26. Pappas, A., Tsiokanos, A., Fatouros, I. G., Poulios, A., Kouretas, D., Goutzourelas, N. and Jamurtas, A. Z. The effects of spirulina supplementation on redox status and performance following a muscle damaging protocol. *International Journal of Molecular Sciences,* **22**(7), 3559 (2021). doi:10.3390/ijms22073559

27. Chen, J. C., Fang, C., Zheng, R. H., Chen, M. L., Kim, D. H., Lee, Y. H. and Bo, J. Environmentally relevant concentrations of microplastics modulated the immune response and swimming activity, and impaired the development of marine medaka Oryzias melastigma larvae. *Ecotoxicology and Environmental Safety,* **241**, 113843, (2022). doi:10.1016/j.ecoenv.2022.113843

28. Saif, G.B. and Khan,I.A. Association of genetic variants of the vitamin D receptor gene with vitiligo in a tertiary care center in a Saudi population: a case-control study. *Annals of Saudi Medicine,* **42**(2), 96-106 (2022). doi:10.5144/0256-4947.2022.96

29. AlThobaiti, S.A. Protective effect Spirulina against Monosodium glutamate-induced hepatic dysfunction: A biochemical, molecular, and histopathological study. *Journal of King Saud University-Science,* **35**(2), 102464, (2023) . doi:10.1016/j.jksus.2022.102464

30. El-Moataaz, S., Ismael, H. and Aborhyem, S. Assessment of chemical composition of Spirulina platensis and its effect on fasting blood glucose and lipid profile in diabetic Rats. *Journal of High Institute of Public Health,* **49**(3), 199-211, (2019) . doi: 10.21608/jhiph.2019.64463

31. Cho, J.A., Baek, S.Y., Cheong,S.H. and Kim, M.R. Spirulina enhances bone modeling in growing male rats by regulating growth-related hormones. *Nutrients,* **12**(4), 1187, (2020) . doi:10.3390/nu12041187

32. Sinha, S., Patro, N. and Patro,I.K. Maternal protein malnutrition: Current and future perspectives of spirulina supplementation in neuroprotection. *Frontiers in Neuroscience,* **12**, 966 (2018), doi:10.3389/fnins.2018.00966

33. Bin-Jumah, M. N., Al-Huqail, A. A., Abdelnaeim, N., Kamel, M., Fouda, M. M., Abulmeaty, M. M. and Abdel-Daim, M. M. Potential protective effects of Spirulina platensis on liver, kidney, and brain acrylamide toxicity in rats. *Environmental Science and Pollution Research,* **28**, 26653-26663(2021), doi: 10.1007/s11356-021-12422-x

34. Mazloomi, S. M., Samadi, M., Davarpanah, H., Babajafari, S., Clark, C. C., Ghaemfar, Z. and Rostami, H. The effect of Spirulina sauce, as a functional food, on cardiometabolic risk factors, oxidative stress biomarkers, glycemic profile, and liver enzymes in nonalcoholic fatty liver disease patients: A randomized double-blinded clinical trial. *Food Science & Nutrition,* **10**(2), 317-328(2022). doi:10.1002/fsn3.2368

35. Abo-Shady, A.M., Gheda, S.F., Ismail, G.A., Cotas, J., Pereira, L. and Abdel-Karim, O. H. Antioxidant and antidiabetic activity of algae. *Life,* **13**(2), 460, (2023). doi:10.3390/life13020460

36. Rezaiyan, M., Najmeh S., Asma K., Mohammad A. Mohsenpour , Siavash B. Seyed M. Mazloomi ., Cain C T Clark., Javad H., Zohreh G. Far , Mohsen A. and Alireza Z.. The effect of spirulina sauce on glycemic index, lipid profile, and oxidative stress in type 2 diabetic patients: A randomized double-blind clinical trial. *Food Science & Nutrition,* **11**(9), 5199-5208 (2023). doi:10.1002/fsn3.3479

37. Rostami, H. A. A., Marjani, A., Mojerloo, M., Rahimi, B. and Marjani, M., Effect of spirulina on lipid Profile, glucose and malondialdehyde levels in type 2 diabetic patients. *Brazilian Journal of Pharmaceutical Sciences,* **58**, e191140 (2022). doi:10.1590/s2175-97902022e191140

38. Hussein, M. M., Samy, M., Arisha, A. H., Saadeldin, I. M. and Alshammari, G. M. Anti-obesity effects of individual or combination treatment with Spirulina platensis and green coffee bean aqueous extracts in high-fat diet-induced obese rats. *All Life,* **13**(1), 328-338 (2020) . doi:10.1080/26895293.2020.1781698

39. Abdella, A., Fouad, M., Irmak,S. and Alamri, E. Hypolipidemic, Antioxidant and Immunomodulatory Effects of Lactobacillus casei ATCC 7469-Fermented Wheat Bran and Spirulina maxima in Rats Fed a High-Fat diet. *Fermentation,* **8**, 610(2022).

40. Anton, C., Ivan, D., Loghin, C., Flutur, N., and Grigore, M. N., Spirulina (Arthrospira) Platensis-A Review Of Possible Hypotriglyceridemic Effects. *Advances in Medical and Biological Sciences,***1**(1),21-32(2023). doi:10.4316/AMBS.2023.1.1.0003

41. Cichoż-Lach, H. and Michalak, A. Oxidative stress as a crucial factor in liver diseases. *World Journal of Gastroenterology: WJG,* **20**(25), 8082(2014). doi: 10.3748/wjg.v20.i25.8082

42. Li, J., Liu, H. and Shang,L. Tert-butylhydroquinone Mitigates Renal Dysfunction in Pregnant Diabetic Rats via Attenuation of Oxidative Stress and Modulation of the iNOs/NFkB/TNF Alpha Signalling Pathway. Endocrine, Metabolic & Immune Disorders-Drug Targets (Formerly Current Drug Targets-Immune.*Endocrine & Metabolic Disorders)*, **23**(5), 633-646(2023). doi:10.2174/1871530322666220908153118

43. Saxena, B. and Sharma,S. Food color induced hepatotoxicity in Swiss albino rats, Rattus norvegicus. *Toxicology International,* **22**(1),152, (2015). doi: 10.4103/0971-6580.172286

44. Rahmanian, N., Shokrzadeh,M. and Eskandani, M. Recent advances in γH2AX biomarker-based genotoxicity assays: A marker of DNA damage and repair. *DNA Repair,* **108**, 103243(2021). doi:10.1016/j.dnarep.2021.103243

45. Javaheri-Ghezeldizaj, F., Alizadeh, A.M., Dehghan, P. and Dolatabadi, J.E.N. Pharmacokinetic and toxicological overview of propyl gallate food additive. *Food Chemistry,* 135219 (2023) doi:10.1016/j.foodchem.2022.135219

46. Abouzed, T. K., Althobaiti, F., Omran, A. F., Eldomany, E. B., El-Shazly, S. A., Alharthi, F. and Dorghamm, D. A. The chemoprevention of spirulina platensis and garlic against diethylnitrosamine induced liver cancer in rats via amelioration of inflammatory cytokines expression and oxidative stress. *Toxicology Research,* **11**(1), 22-31 (2022) . doi:10.1093/toxres/tfab118

47. Alexander-Dann, B., Pruteanu, L. L., Oerton, E., Sharma, N., Berindan-Neagoe, I., Módos, D. and Bender, A. Developments in toxicogenomics: understanding and predicting compound-induced toxicity from gene expression data. *Molecular Omics,* **14**(4), 218-236 (2018).doi: 10.1039/C8MO00042E

48. Fowler, P., Meurer, K., Honarvar,N. and Kirkland, D. A review of the genotoxic potential of 1, 4-naphthoquinone. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis,* **834**, 6-17 (2018) . doi:10.1016/j.mrgentox.2018.07.004

# تأثير الطحالب الدقيقة ومركباتها النشطة بيولوجيا على صحة الكبد في الجرذان المعرَّضة لمضادات الأكسدة الفينولية الإصطناعية

**سارة محمود عمر [1]، راشد الحويتان [2]، السيد عثمان حسين [3]، برانسيلي جاليك [4]، ناصر نسيم زهران[5] ، محمود العجواني[6] و أحمد علي صالح [7]***

[1]قسم التغذية وعلوم الأطعمة– كلية الاقتصاد المنزلي– جامعة الأزهر – طنطا – مصر.

[2]قسم الانتاج الحيواني– كلية الأغذية والزراعة– جامعة الملك سعود– الرياض– السعودية.

[3]المجموعة الخماسية للأعلاف والمنتجات الحيوانية– الرياض– السعودية.

[4]مركز التغذية والجينوم– كلية الزراعة في نترا– نترا– سلوفاكيا.

[5]قسم التغذية – مستشفيات جامعة المنوفية– شبين الكوم – مصر.

[6] قسم انتاج الدواجن– كلية الزراعة– جامعة الزقازيق– مصر.

[7]قسم انتاج الدواجن– كلية الزراعة– جامعة كفرالشيخ– مصر.

تهدف هذه الدراسة إلي دراسة ما إذا كان مسحوق الإسبيرولينا يمكن أن يساعد في حماية الجرذان من التأثيرات الضارة لثلاثي بوتيل هيدروكينون. وقد تم تقسيم حيوانات التجربة إلى أربع مجموعات، تضم كل منها سبعة جرذان. تتألف المجموعة الأولى من جرذان عادية كمجموعة ضابطة سالبة، بينما تعرضت المجموعة الثانية لجرعة قدرها 100 ملجم/كجم (وزن الجسم) من ثلاثي بوتيل هيدروكينون لمدة 7 أيام كمجموعة ضابطة موجبة. فى حين تلقت المجموعة الثالثة جرعة قدرها 500 ملجم/كجم (وزن الجسم) من مسحوق الإسبيرولينا و100 ملجم/كجم من ثلاثي بوتيل هيدروكينون، وأخيراً تم إعطاء المجموعة الرابعة خليطًا من 750 ملجم/كجم من مسحوق الإسبيرولينا و100 ملجم/كجم من ثلاثي بوتيل هيدروكينون. كان لدى الجرذان التي أعطيت ثلاثي بوتيل هيدروكينون وحده مستويات أعلى بكثير من إنزيمات الكبد مثل ALT و AST ونسبة AST/ALT و ALP في دمها مقارنة بالمجموعة الضابطة السالبة. علاوة على ذلك، أظهرت دراسة عينات الأنسجة إلى أن مستويات مضادات الأكسدة من الجلوتاثيون وسوبر أكسيد ديسميوتاز، وإجمالي القدرة المضادة للأكسدة والكتاليز انخفضت بشكل ملحوظ في الجرذان التي تعرضت للسمية الناجمة عن ثلاثي بوتيل هيدروكينون، كما ارتفعت مستويات المالونديالدهيد بشكل ملحوظ. في التجربة، تم إعطاء الجرذان ثلاثي بوتيل هيدروكينون كمجموعة إيجابية، مما أدى إلى زيادة في دلائل دهون الدم مثل الكوليستيرول الكلى والدهون الثلاثية والليبوبروتين منخفض الكثافة والليبوبروتين منخفض الكثافة جداً باستثناء الليبوبروتين عالى الكثافة بالمقارنة مع المجموعة الضابطة السالبة. أدى تناول مسحوق الإسبيرولينا إلى تعزيز المعلمات البيوكيميائية في الدم وانخفاض مستويات المالونديالدهيد في الجرذان التي أصيبت بمادة ثلاثي بوتيل هيدروكينون كمجموعة إيجابية، علاوة على ذلك، أظهرت الجرذان زيادة في مؤشرات مضادات الأكسدة لديها. كما تُظهر نتائج الدراسة الذي تم اجراؤها إلي وجود صلة قوية بين جينات β-actin و Bcl2 و HO-1 و Nrf2 في كبد المجموعات الأربعة من الجرذان التي خضعت للتجربة. ويمكن التخليص أن هناك تأثير وقائي قوي وذو دلالة إحصائية لتاثير مسحوق الإسبيرولينا على الجرذان، وقد شوهد هذا التأثير في دلائل الدهون في الدم، وعلامات مضادات الأكسدة، وانزيمات الكبد بعد التعرض لمادة ثلاثي بوتيل هيدروكينون كمثال لمضادات الأكسدة الفينولية الإصطناعية.

**الكلمات الدالة:** ثلاثى بيوتيل هيدروكينون – اسبيرولينا بلاتنسيس – دلائل الدهون– دلائل مضادات الأكسدة– التعبير الجينى – الكبد.