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Impact of Using Spirulina Solution on Growth, Blood Biochemistry, and Redox Balance in Dogs as an Oral Supplement

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Abstract

SPIRULINA, a blue-green microalga, has garnered attention for its potential benefits in animal health due to its rich nutrient profile, including proteins, vitamins, minerals, and antioxidants. This study explores how Spirulina Platensis (SP) can enhance animal health. It focuses on its effects on body weight, hematobiochemical characteristics, and antioxidant status in dogs after in-vitro pharmacological analysis of Spirulina cytotoxicity and efficacy. Fifteen recently recovered dogs from tick infestation with average age (3-5 months) and weight (3-4 kg) were assigned to this experimental study and given 2.5 ml of SP (4%) oral solution 15 minutes before morning feed. The experiment lasted 45 days, and each animal's body weight and blood samples were obtained on days 0, 15, 30, and 45. Hematology and serum biochemistry (lipid profile, liver enzymes, renal function, and oxidant /antioxidant state) were evaluated. HPLC analysis for the SP was performed and revealed 10 molecules of polyphenols where gallic acid was the highest predominant molecule. The results revealed that SP supplementation enhanced live body weight gain, increased WBCs, and antioxidant activity, and liver enzymes, and oxidative markers at day 15, 30, and 45 with decreased in triglycerides, and LDL-C between 15 and 45 days with non-significant decrease in cholesterol level compared to the zero days (pre-SP supplementation). The current study's data suggest that SP supplementation should be added to dogs' meals to improve growth performance, antioxidant capacity, and general health.Keywords: Meat quality, Cerium oxide NPs, Zinc oxide NPs, Epididymal sperms, Ram.

Keywords: Dogs, Spirulina platensis, Body weight, Antioxidants, Lipid profile.

Introduction

One health problem that is of great concern, especially in developing countries, is malnutrition. Severe forms of malnutrition are expressed as protein energy malnutrition defects. A total healthy food which includes vitamins and minerals that is essential for healthy growth and development. The value of poor nutrition is a major problem, which affects survival as well as death rate. Spirulina offers an ease, excellent and worldwide supplementation source of a lot of macro- and micronutrients which is highly important to human health [1].

Spirulina is a prokaryote microorganism of the cyanobacteria phylum [2] which is blue - green algae due to the presence of both chlorophyll (green) and phycocyanin (blue) pigments in its cellular structure. The powerful effects of spirulina as a nutritional supplement which benefit your health with special focus on its immunomodulatory and antioxidant effects [1]. The high nutritional profile of Spirulina includes a protein content reaching 60–70% of dry weight with a profile that includes all the essential amino acids for dogs [3]. Spirulina is rich in proteins, carbohydrates, polyunsaturated fatty acids, sterols and some more vital elements such as calcium, iron, zinc, magnesium, manganese and selenium. It is a natural source of vitamin B12, vitamin E, ascorbic acid, tocopherols and a whole spectrum of natural mixed carotene and xanthophylls phytopigments.

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Spirulina as a supplement serves to provide these nutrition requirements and seems to be a 'wonder food'[1].

Spirulina also, has an important role in controlling diabetes with significant decrease in plasma triglycerides (total- and LDL-cholesterol), maintaining normal blood pressure with an improvement in antioxidant status, along with anti-inflammatory effect [4].

The spirulina solution was proved to have a great impact on the immunity by upregulating the phagocytic activity of macrophages, stimulating the natural killer cells. It also enhances the activation and mobilization of T and B cells in response to the production of cytokines and antibodies [5].

There is no paper discuss the effect of SP after tick infestation but it is known that ticks can compromise the immune system and expose dogs to various pathogens so using SP in dogs after tick infestation can enhance immunity of dogs.

A phytochemical abundantly present in spirulina, phycocyanobilin reportedly hinders NADPH oxidase activity and encourages glutathione production along with a massive increase in the synthesis of antioxidant enzymes having a significant effect in management of oxidative stress in sickle cell disease [6]. As an antioxidant, it contains both enzymatic (superoxide dismutase, glutathione peroxidase, catalase, ascorbate peroxidase) and non-enzymatic (carotenoids, ascorbic acid, tocopherols, chlorophyll derivates) antioxidant protection system [7].

The nutraceutical and therapeutical applications of Spirulina in pet animals are thus largely unexplored. That is why, no previous references are available on the recommended dose of Spirulina needed to accomplish health benefits in dogs and cats, and at the same time the possible risk of side effects is not yet identified. Despite the shortage of experimental trials on the possible effects of the inclusion of Spirulina in dog and cat food, its use has been increased in the pet food market in latest years [3].

Spirulina, enhances growth performance as it is packed with essential amino acids, vitamins (B-complex, C, and E), and minerals (iron, magnesium, and potassium), which provide the necessary nutrients for growth and development. It contains a high percentage of proteins (50–70%) that aid in muscle and tissue building [8, 9].

It also improved gut health that enhances nutrient absorption, contributing to overall growth and performance. In addition, Spirulina is rich in phycocyanin, beta-carotene, and other bioactive compounds with antioxidant properties, which protect cells from oxidative stress, reduced oxidative damage, enhances cellular function, and promoting better growth [10].

Nevertheless, it was proposed that CAT, SOD, and GSH are critical endogenous antioxidant molecules that protect against free radicals and oxidative damage. All of these enzymes are required for antioxidant equilibrium and homeostasis. SP might be one of the most essential herbal foods and an antioxidant supplement for homeostasis. It is also suggested that due to Spirulina's antioxidant capabilities, dosages must be determined [11, 12].

We aimed to evaluate the beneficial effects of spirulina in dogs as an antioxidant, improve lipid metabolism, cholesterol-lowering activity, and improve health body condition.

This study aims to assess the positive effects of spirulina in dogs, specifically its antioxidant properties, its ability to improve lipid metabolism, lower cholesterol levels, and enhance overall body condition.

Material and Methods

In Vitro Experiment

Preparation Spirulina platensis aqueous extract

Desert Research Center of Egypt provided the dried powder of Spirulina platensis." The experiment was performed at October 2024." As described by [13], an aqueous extract of S. platensis was prepared by mixing equal quantities of dried powder with distilled water. For 1 h, S. platensis was extracted with water in an autoclave at 120 °C. In order to adjust the pH to 4.0, citric acid was added to the hot aqueous extract, which was centrifuged at 2500 RPM for 15 minutes to remove the insoluble fractions. To prepare fresh aqueous extracts for use during the experiment, they were prepared as needed.

Determination of cytotoxicity of test sample

To test the cytotoxicity of SP on cell viability of Wi-38 cells. Wells containing tissue culture medium in the plate were inoculated with 1×10^5 CFU/mL and incubated at 37°C for a day. The growth medium was collected from the wells after the appearance of a monolayer, and then washed twice with sterile distilled water. Double dilution was performed in RPMI medium twice in the presence of 2% serum. Each dilution fraction (0.1 mL) was analyzed in all wells except 3 wells which were control [14]. After incubation of the plate at 37°C for a day, cytotoxicity was tested to detect abnormalities compared to controls [15]. The wells were supplemented with 20 µL of MTT solution and incubated in a shaking incubator at 37°C and 150 rpm for 5 min, then incubated again at 37°C and 5% CO_2 for 4 h. The medium was disposed and the plate was dried using sterile tissue to remove impurities. The reaction product was supplemented with 200 µL of DMSO and incubated in a shaking incubator at 37°C and 150 rpm for 5 min, and then the absorbance was measured at 560 nm [16].

HPLC analysis

The sample extract was analyzed using HPLC (Agilent 1260) with a C8 column. The mobile phase consisted of

water and 0.05% trifluoroacetic acid in acetonitrile at a flow rate of 0.9 mL/min, which was used in a linear gradient, 0 min (82% water), 0-1 min (82% water), 1-11 min (75% water), 11-18 min (60% water), 18-22 min (82% water), 22-24 min (82% water). The multi-wavelength detector was set at 280 nm. The column was injected at 40°C with 5 µL of sample solution [17].

Assay of total saponin content (TSC)

TSC was assayed using the modified vanillin-sulphuric acid method [18]. A natural mixture of triterpenoid saponins (aescin) was used to generate a standard curve. A stock solution of aescin (15 mg/mL) was prepared by dissolving 150 mg of aescin in 10 mL of methanol, and was serially diluted in triplicate with methanol. Methanol was used as a blank solvent for the standards. The extract was prepared by mixing 1 g of powder with 30 ml of ethanol in a 100 mL Erlenmeyer flask. The mixture was left for 30 min before filtration at room temperature, rapidly cooled in ice water, and filtered through a 0.45 membrane filter. The tubes were placed in a water bath at 65°C for 5 min to allow the methanol to evaporate to dryness. 0.5 mL of 4% vanillin in ethanol (w/v) was added to each tube, followed by 2.5 mL of 72% H_2SO_4 (v/v). The tubes were capped, mixed, incubated in a water bath at 60°C for 15 min, and cooled for 5 min in water at room temperature. The absorbance of the solutions was measured at 560 nm using a Biosystem 310 spectrophotometer after using the blank sample to calibrate the instrument. Absorbance values were plotted against concentrations to construct a standard curve. TSC was calculated using the following equation:

$$\text{TSC mg aescin/g} = \frac{\text{weight of saponin in the extract (mg)}}{\text{weight of powder (g)}} \times 1 - (\text{moisture \%}/100)$$

Assay of total tannin content (TTC)

TTC was assayed using the acidified vanillin method [19], where tannic was used as a standard. The extract (400 µL) was added to 3 mL of vanillin solution (4% in methanol) and 1.5 mL of concentrated HCl. After 15 min of incubation, the absorbance was measured at 500 nm.

Assay of total terpenes content (TTPC)

The plant extract (10 mg) was dissolved in 95% methanol, and 200 µL was withdrawn into a 2 mL test tube and added to 1.5 mL of chloroform. A solution of limonene in methanol (200 µL) was added to 1.5 mL of chloroform and serially diluted (100 mg/mL to 5 mg/mL) to generate a standard curve. The mixture was vortexed and left for 3 min. Sulfuric acid (100 µL) was added to each 2 mL test tube, and the mixture was cooled in ice water for 15 min to avoid the harmful effect of the generated heat. The mixture was incubated in a dark at room temperature for 2 h. The linalool standard was incubated for 5 min, a reddish-brown precipitate formed at the end of incubation of the standard and sample. Since the reddish-brown precipitate was partially soluble in the reaction mixture, the supernatant was gently decanted. The mixture was supplemented with 1.5 mL of 95% (v/v) methanol and vortexed thoroughly until the precipitate was completely dissolved in the methanol. The absorbance was measured at 546 nm using a Biosystem 310 plus spectrophotometer, and methanol was used as a blank. TTPC was calculated as limonene equivalents using the regression equation of the limonene standard curve [20].

Fourier Transform Infrared (FTIR) analysis

FTIR spectroscopy technique was used to identify the chemical groups present in the compound. The tested sample was scanned using FTIR to determine the chemical properties. The FTIR device emitted infrared radiation at 10,000 cm^{-1} to 100 cm^{-1} through the tested sample, where the sample molecules enhanced the conversion of the absorbed radiation into rotational and/or vibrational energy. The signal appeared in the detector as a spectrum (4000 cm-1 to 400 cm-1) which formed a molecular fingerprint of the sample [21].

In Vivo Experiment

Ethical approval

Our experimental design, and methodology was done according to the guidelines and regulations of the Institutional Animal Care and Use committee of the Cairo University CU-IACUC (Vet CU131020241014).

Our current study protocol and methodology is reported in accordance with ARRIVE guidelines.
Animals

Fifteen recently recovered dogs from tick infestation were assigned to our experimental trial, housed in private kennels at small animal teaching hospital, which belong to Department of Medicine and Infectious Diseases, Cairo University, Egypt. Animals' age was (3-5 months) with average weight (3-4 kg). They drenched daily by amount of 2.5 ml of Spirulina platensis aqueous extract with 4% concentration 15 minutes before morning feeding with commercial dry food. Water was available all the day. The experiment last for 45 days, body weight was measured and samples were taken from each animal at day 0, 15, 30 and 45 of the experimental trial.

Blood Samples

Blood is drawn from each dog's cephalic vein using a 22 gauge needle on EDTA containing tubes for hematological analysis, and plain tubes for serum separation to analyze the antioxidants, and blood biochemistry.

Hematological analysis was performed using an automated hematological analyzer (Abaxis, Zoetis Vetscan® HM5, USA).

Blood biochemistry including liver enzymes; alanine aminotransferase (ALT), and aspartate aminotransferase (AST), kidney function tests; blood urea nitrogen (BUN), and creatinine (Cr) using kits of (Spectrum Company, Egypt), total cholesterol (TC), and low density lipoprotein cholesterol (LDL-C), and triglycerides using Biochemistry Analyzer Pictus B Diatron as detailed in [22].

Antioxidant activity were analyzed using special ELISA kits (MyBioSource, USA) of superoxide dismutase (SOD), and glutathione peroxidase (GSH-PX), furthermore malondialdehyde (MDA) and catalase (CAT) were analyzed through (BIO-DIAGNOSTIC, Egypt) ELISA kits as referred by [23].

Analysis of statistical data

Based on the illustrated data, the data were analyzed using ANOVA test where the mean and SEM are expressed as Mean ±SEM with SPSS 27 (IBM, New York, New York, USA). Means having similar lowercase letter within the same row are not significantly different at *P* value less than 0.05 level of probability.

In Vitro Experiment

Sample viability and cytotoxicity were tested on WI38 cell line (lung tissue) as represented in Table 1. The normal cell line is considered free of abnormalities (Figure 1a). Different concentrations of the sample (1000 µg/mL, 500µg/mL, 250µg/mL, 125µg/mL, 62.5µg/mL, and 31.25µg/mL) were tested for their cytotoxicity as shown in Figure 1b, Figure 1c, Figure 1d, Figure 1e, Figure 1f, and Figure 1g respectively. Low cytotoxicity of the sample in lung tissue (WI38 cell line) was detected with IC_{50} of 305.2 µg/mL as shown in (Figure 2). The sample showed 90.1% toxicity and 9.8% viability at 1000 µg/mL. Toxicity was found to be directly proportional to sample concentration and inversely proportional to viability.

HPLC Analysis

The methanolic extract of polyphenols as a standard was analyzed using HPLC as summarized in Figure 3. Gallic acid was detected as the first peak with a retention time of 3.572, a width of 0.0890 min, and an area of 320.66037 mAU*s for 3.9105%. Chlorogenic acid was detected as the second peak with a retention time of 4.236, a width of 0.1281 min, and an area of 465.45001 mAU*s for 5.6762%.

Catechin was detected as the third peak with a retention time of 4.450, a width of 0.1451 min, and an area of 413.51682 mAU*s for 5.0429%. Methyl gallate was detected as the fourth peak with a retention time of 5.424, a width of 0.1617 min, and an area of 360.46564 mAU*s for 4.3959%. Coffeic acid was detected as the fifth peak with a retention time of 5.860, a width of 0.1678 min, and an area of 285.98538 mAU*s for 3.4876%.

Syringic acid was detected as the sixth peak with a retention time of 6.351, a width of 0.1709 min, and an area of 320.92712 mAU*s for 3.9137%. Rutin was detected as the seventh peak with a retention time of 6.883, a width of 0.1734 min, and an area of 399.98840 mAU*s for 4.8779%. Ellagic acid was detected as the eighth peak with a retention time of 7.219, a width of 0.2281 min, and an area of 705.82501 mAU*s for 8.6076%.

Coumaric acid was detected as the ninth peak with a retention time of 8.569, a width of 0.1897 min, and an area of 723.95392 mAU*s for 8.8287%. Vanillin was detected as the tenth peak with a retention time of 8.948, a width of 0.2176 min, and an area of 440.50281 mAU*s for 5 5.3720%. Ferulic acid was detected as the eleventh peak with a retention time of 9.610, a width of 0.1977 min, and an area of 445.93845 mAU*s for 5.4382%. Naringenin was detected as the twelfth peak with a retention time of 10.288, a width of 0.2096 min, and an area of 414.13177 mAU*s for 5.0504%.

Rosmarinic acid was detected as the thirteenth peak with a retention time of 11.680, a width of 0.2122 min, and an area of 604.53595 mAU*s for 7.3724%. Daidzein was detected as the fourteenth peak with a retention time of 15.834, a width of 0.2349 min, and an area of 405.05698 mAU*s for 4.9397%. Querectin was detected as the fifteenth peak with a retention time of 17.180, a width of 0.2165 min, and an area of 301.81699 mAU*s for 3.6807%. Cinnamic acid was detected as the sixteenth peak with a retention time of 19.080, a width of 0.1994 min, and an area of 704.45416 mAU*s for 8.5909%. Kaempferol was detected as the seventeenth peak with a retention time of 20.519, a width of 0.1879 min, and an area of 358.23932 mAU*s for 4.3687%. Hesperetin was detected as the eighteenth peak with a retention time of 21.095, a width of 0.1801 min, and an area of 528.59406 mAU*s for 6.4462%. The total turns out to be 8200.04315.

The methanolic extract of the sample was analyzed using HPLC compared to polyphenols as a standard (Figure 4). The methanolic extract consisted of different concentrations of eighteen components was represented in (Table 2). Gallic acid was detected as the first peak with a retention time of

3.525, a width of 0.1510 min, and an area of 88.21975 mAU*s for 57.4632%. Chlorogenic acid was detected as the second peak with a retention time of 4.165, a width of 0.2156 min, and an area of 35.28670 mAU*s for 22.9845%. Catechin was detected as the third peak with a retention time of 4.449, a width of 0.0000 min, and an area of 0.0000 mAU*s for 0.0000 %.

Methyl gallate was detected as the fourth peak with a retention time of 5.541, a width of 0.1230 min, and an area of 1.80478 mAU*s for 1.1756%. Coffeic acid was detected as the fifth peak with a retention time of 5.847, a width of 0.1393 min, and an area of 1.71751 mAU*s for 1.1187%. Syringic acid was detected as the sixth peak with a retention time of 6.553, a width of 0.2601 min, and an area of 13.17472 mAU*s for 8.5815%.

Rutin was detected as the seventh peak with a retention time of 6.910, a width of 0.0000 min, and an area of 0.0000 mAU*s for 0.0000 %. Ellagic acid was detected as the eighth peak with a retention time of 7.222, a width of 0.0000 min, and an area of 0.0000 mAU*s for 0.0000%. Coumaric acid was detected as the ninth peak with a retention time of 8.285, a width of 0.1746 min, and an area of 2.24658 mAU*s for 1.4633%.

Vanillin was detected as the tenth peak with a retention time of 8.958, a width of 0.2533 min, and an area of 3.91827 mAU*s for 2.5522%. Ferulic acid was detected as the eleventh peak with a retention time of 9.828, a width of 0.2167 min, and an area of 1.85423 mAU*s for 1.2078%. Naringenin was detected as the twelfth peak with a retention time of 10.318, a width of 0.0000 min, and an area of 0.0000 mAU*s for 0.0000 %. Rosmarinic acid was detected as the thirteenth peak with a retention time of 11.890, a width of 0.2390 min, and an area of 3.68745 mAU*s for 2.4019%. Daidzein was detected as the fourteenth peak with a retention time of 15.067, a width of 0.1773 min, and an area of 1.61386 mAU*s for 1.0512%.

Querectin was detected as the fifteenth peak with a retention time of 17.202, a width of 0.0000 min, and an area of 0.0000 mAU*s for 0.0000%. Cinnamic acid was detected as the sixteenth peak with a retention time of 19.095, a width of 0.0000 min, and an area of 0.0000 mAU*s for 0.0000%. Kaempferol was detected as the seventeenth peak with a retention time of 20.538, a width of 0.0000 min, and an area of 0.0000 mAU*s for 0.0000%. Hesperidin was detected as the eighteenth peak with a retention time of 21.117, a width of 0.0000 min, and an area of 0.0000 mAU*s for 0.0000%. The total turns out to be 153.52384.

Phytochemical Screening

Saponins, tannins, and terpenes were detected as major components of the methanolic extract of the sample as shown in (Table 3). Saponins showed the highest concentration of 61.33 mg/g, followed by tannins and terpenes at 49.93 mg/g and 49.33 mg/g, respectively. The absorbances of a wide range (5 µg/mL, 10 µg/mL, 20 µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL, 100 µg/mL) of limonene as a terpene standard were measured at 546 nm and showed of 0.082, 0.177, 0.275, 0.496, 0.791, 0.994, and 1.199, respectively (Figure 5a).

The absorbances of a wide range (0.625 µg/mL, 1.25 µg/mL, 2.5 µg/mL, 5.0 µg/mL, 10 µg/mL) of aescin as a saponin standard were measured at 560 nm and showed of 0.11, 0.214, 0.392, 0.783, and 1.566, respectively (Figure 5b). The absorbances of a wide range (10 µg/mL, 20 µg/mL, 40 µg/mL, 80 µg/mL, 100 µg/mL) of tannic acid as a tannin standard were measured at 500 nm and showed of 0.141, 0.382, 0.695, 1.266, and 1.593, respectively (Figure 5c). The absorption of terpenes, saponins and tannins was found to increase directly with their concentration until it reached its peak at 1.119, 1.566 and 1.593, respectively. Therefore, the methanolic extract of the sample contained tannins as the highest component, followed by saponins and terpenes.

Fourier Transform Infrared (FTIR) analysis

The methanolic extract of the sample was analyzed using FTIR (Figure 6). The FTIR spectrum showed different peaks with different characteristics. The first peak appeared in the frequency range from 4000 cm^{-1} to 3000 cm^{-1} and the absorption of 3442.81 cm^{-1} which lies between 3200 cm^{-1} and 3600 cm^{-1} as a strong and broad peak indicating the O-H stretching group of the alcohol bonded intermolecularly and phenols. The second peak appeared in the frequency range from 2400 cm^{-1} to 2000 cm^{-1} and the absorption of 2076.35 cm^{-1} located between 1800 cm^{-1} and 2400 cm^{-1} as a strong peak indicating the N=C=S stretching group of isothiocyanate. The third peak appeared in the frequency range from 1670 cm^{-1} to 1600 cm^{-1} and the absorption of 1635.78 cm^{-1} located between 1750 cm^{-1} and 1440 cm^{-1} as a medium peak indicating the C=C stretching group of cyclic alkenes.

The fourth peak appeared in the frequency range from 1600 cm^{-1} to 1300 cm^{-1} and the absorption of 1479.75 cm^{-1} located between 1490 cm^{-1} and 1390 cm^{-1} as a medium peak indicating the C-H bending group of the methyl alkane group. The fifth peak appeared in the frequency range from 1600 cm^{-1} to 1300 cm^{-1} and the absorption of 1442.69 cm^{-1} located between 1470 cm^{-1} and 1380 cm^{-1} as a medium peak indicating the C-H bending group of the methyl alkane group. The sixth peak appeared in the frequency range from 1600 cm^{-1} to 1300 cm^{-1} and the absorption of 1405.63 cm^{-1} located between 1450 cm^{-1} and 1340 cm^{-1} as a medium peak indicating the O-H bending group of the methyl alkane group.

The seventh peak appeared in the frequency range from 1400 cm^{-1} to 1000 cm^{-1} and the absorption of 1083.74 cm^{-1} located between 1100 cm^{-1} and 900 cm^{-1} as a strong peak indicating the C-O stretching group of the primary alcohol. The eighth, ninth, and tenth peaks with absorptions of 538.30 cm^{-1}, 469.84 cm^{-1}, and 438.48 cm^{-1}, respectively, appeared as insignificant peaks because they are located below the frequency of 600 cm^{-1} indicating a halo compound. Therefore, some of the proposed functional groups were found to enter into the structure of the compound. The O-H stretching group indicates the presence of alcohols and phenols, the N=C=S stretching group indicates the presence of isothiocyanates in the form of nitriles, the C=C stretching group indicates the presence of cycloalkenes, the C-H bending group indicates the presence of an aldehyde, and the C-O stretching group indicates the presence of alcohols and phenols. Thus, this compound contains alcohols, phenols, nitriles, cycloalkenes, and aldehydes.

In Vivo Experiment

As detailed in Table 4; no statistical significant differences in hematology parameters, whereas there were significant increases in WBCs count, neutrophils, lymphocytes, BUN, and triglycerides between 0 and 30 days post spirulina supplementation, with significant increase in lymphocytic count and BUN level between 15 and 30 days post supplementation, beside significant increase in monocytes, and triglycerides between 0 and 15 days post supplementation. Weight also, showed significant improvement in 15, 30, and 45 days compared to 0 days.

Significant increase were noticed in WBCs count and neutrophils between 30 and 45 days, monocytes, AST, triglycerides, and LDL-C between 15 and 45 days, AST, ALT, and LDL-C between 0 and 45 days with decrease in AST, and ALT between 0 and 30 days along with decreases in AST, and monocytes between 0 and 15 days, and 15 and 30 days, respectively post oral spirulina supplementation Table 4.

Anti-oxidant activity were greatly influenced by spirulina supplementation as CAT, and SOD showed significant increase in 15, 30, and 45 days compared to 0 day, and between 30 and 45 days post supplementation with increase in CAT, and GSH in 30 and 45 days compared to 15 days, beside an increase in GSH in 30 and 45 days compared to 0 day post supplementation. While MDA showed significant decrease in 15, 30, 45 days compared to 0 day and decrease in 30 and 45 days compared to 15 days post supplementation along with significant decrease in SOD level in 30 days compared to 15 days as tabulated in Table 5.

Discussion

Spirulina platensis (SP) is small blue - green alga that is regarded as one of the greatest sources of organic ingredients, making it an excellent nutritional supplement for human and animal feed globally. Spirulina was recently classified as a genus of photosynthetic bacteria, Arthrospira [24]. Spirulina includes high-quality proteins, vitamins, and minerals, as well as a diverse range of natural carotene and xanthophyll phytopigments. These ingredients give spirulina a unique and exceptional nutrient composition that allows it to be utilized as a dietary supplement not only to improve nutritional quality but also for therapeutic purposes [25].

Regarding to our results of cytotoxicity, [26] reported that *Spirulina* extract with 70% ethanol showed severe cytotoxicity in K562 and Kasumi-1 cell lines with IC_{50} of 4.64 mg/mL and 3.68 mg/mL, respectively, using trypan blue solution. However, using MTT assay, *Spirulina* extract with 70% ethanol showed less cytotoxicity in K562 and Kasumi-1 cell lines with IC_{50} of 0.40 mg/mL and 0.31 mg/mL, respectively. Furthermore, the *Spirulina* extract with water showed less cytotoxicity than that of the ethanolic extract in K562 and Kasumi-1 cell lines with IC_{50} of 12.68 mg/mL and 2.13 mg/mL, respectively, using trypan blue solution. However, using MTT assay, *Spirulina* extract with water showed less cytotoxicity in K562 and Kasumi-1 cell lines with IC_{50} of 15.77 mg/mL and 9.44 mg/mL, respectively [27].

In the present investigations SP extract has been tested on Wi-38 cells to assure its potency. Similar studies have been examined the cytotoxic effects of the molecules in SP on normal and tumor cells [28, 29]. Compounds. Also, [30] have been tested algal extracts on both malignant and normal cells including lung cells.

Regarding to HPLC results, [31] reported that *S. platensis* extract contains various nutrients with high nutritional values, including fatty acids and minerals, as well as other components with antimicrobial activities. HPLC analysis of *S. platensis* extract showed the presence of nine fatty acids with C18:3 linoleic acid (omega-3) and C18:1 oleic acid in 23.734% and 17.159%, respectively. Furthermore, atomic absorption spectrometry demonstrated that S. platensis extract contained fourteen minerals, with magnesium and iron observed at the highest concentrations of 6548 µg/g and 1250 µg/g, respectively. Environmental factors and algal species directly influence the variation in fatty acid content and the variation in mineral concentrations apparently in relation to the composition of the host soil [32, 33]. Concentrations and extraction time, as well as climatic conditions, affect the diversity and yield of fatty acids and minerals [34].

Various organic solvents are effectively used in the extraction of *Spirulina*, including distilled water, ethanol, ethyl acetate, petroleum ether, hexane, and chloroform. Regarding to phytochemical results, the *spirulina* extract from these organic solvents showed a wide variety of active components, including saponins, phenols, tannins, and terpenoids. Petroleum ether was the most suitable organic solvent from which saponins, phenols, tannins and terpenoids were extracted abundantly. On the other hand, distilled water, ethanol and ethyl acetate were less suitable organic solvents for *Spirulina* extraction, as some active components were not extracted [35].

Regarding to FTIR analysis, [36] reported that FTIR spectroscopy was used to analyze *Spirulina* extract, *Spirulina* powder, and dextrin standard. FTIR spectra showed that protein (1 657 cm^{-1} and 1 537 cm^{-1}) and carbohydrate (1 069 cm^{-1} and 1054 cm^{-1}) were the main features of the fingerprint. In *spirulina* powder, protein and carbohydrate were the predominant nutrients, followed by dextrin.

SP supplemented dogs had considerably higher live body weights ($P<0.05$) compared to the zero day (pre-SP supplementation). These findings are consistent with earlier studies showing that SP supplementation increased live weights in cattle [37], sheep [38], and lambs [39-41]. Spirulina's high nutritional density and promotion of extracellular enzyme production by gut microbiota may contribute to improved growth performance in dogs fed SP-supplemented diets [42].

Supplementing spirulina orally daily in a water suspension at a 1:10 w/v ratio can increase both performance and cost efficiency for lamb farmers [43]. Spirulina includes a variety of nutrients, including vitamins, minerals, vital fatty acids, amino acids, and other elements that may stimulate quicker development [44]. As reported by [45] who found that adding spirulina platensis to feed boosted palatability and consequently feed efficiency. Spirulina platensis exhibited a noticeable stimulating impact on metabolism during the period of rapid growth of calves' organs, which might explain the improvement in body weight [46]. Spirulina might also promote animal development by enhancing feed intake, feed conversion, nutrient absorption and utilization and body weight gain [47, 48].

Spirulina as a feed supplement (5g/kg feed) might be used safely to increase the development and health of ram lambs [49] and goats (2 to 4g Spirulina/head/day) [50]. As concluded by [51, 52] that adding spirulina (0.5 and 1g/10kg body weight) to sheep's feed improved their health and growth rate.

The assessment of hematological parameters can be used to understand how a chemical substances or plant extracts work in an organism. Blood serves as a pathological indicator of the health of animals exposed to toxins and other circumstances and/or agents [53]. Spirulina contains phycocyanine, which has the ability to promote cell creation inside bone marrow, resulting in the stimulation of white blood cell and platelet synthesis, which is required for life [54].

In terms of hematological measures, Hb%, RBC count, HCT%, and RBCs indices showed no significant decline, while platelets count showed no significant rise in SP supplemented dogs and remained within normal range when compared to zero days. These findings were consistent with [55], who found that spirulina had no influence on blood parameters in rabbits. Also, [41] discovered that SP supplementation as a feed additive for lambs reduced the number of red blood cells. Our findings, however, contradicted those of [11, 39, 46], who showed a substantial rise in hemogram with the addition of spirulina.

White blood cells count, neutrophils, lymphocytes, and monocytes increased significantly in SP-supplemented dogs as compared to the zero days. Leucocytes play a crucial role in non-specific or innate immunity, and their count can indicate decreased disease susceptibility [56]. Spirulina's phycocyanin and polysaccharide components may be responsible for enhanced WBCs production [57]. Spirulina polysaccharide supplementation boosted WBCs counts in mice (at a dosage of 30-60 mg/kg), dogs (at a dose of 12 mg/kg) [58], rabbits [11], and lambs [39, 41]. Spirulina was also observed to improve immunity in hens given 10 g/kg of Spirulina platensis [59] and fish [60]. This implies that spirulina supplements may stimulate the immune system. A polysaccharide extract of SP has been found in studies to boost white blood cells production in mice and dogs with an irradiated hematopoietic system, which is responsible for blood cell synthesis [58].

Also, [41, 61] provide more support for these findings. In their investigation, mice treated with Spirulina platensis algae showed a considerable rise in the number of white blood cells as well as enhanced activity of macrophages. These findings imply that Spirulina platensis has the ability to boost the immune system. The methods by which spirulina impacts white blood cell counts and red blood cell characteristics are not yet fully known, however it is possible that the physiologically active components in spirulina increase white blood cell formation or activity, particularly at larger dosages. However, further study is needed to determine the exact cell types and signaling mechanisms at work. Also, [41] reported that SP supplementation significantly improved lambs' growth performance and immune response, as evidenced by increased weight gain and feed intake, as well as higher white blood cell counts.

In terms of cholesterol levels and LDL-C, it resulted in a reduction on all days following SP supplementation. This condition indicates that Spirulina is a lipid-lowering agent, as validated by [62]. These findings are also consistent with those of [63], who found that Spirulina platensis causes a considerable drop in total cholesterol levels in rabbits fed a high-cholesterol diet.

Supplementing with SP lowered total plasma cholesterol. This is similar with prior research in rats [64], hamsters [65], rabbits [11, 66], humans [67], and lambs [39]. Although the mechanism by which the SP reduces cholesterol has not been fully examined, the hypocholesterolemic actions of SP involve reducing plasma and liver cholesterol levels due to an increase in lipoprotein lipase and hepatic triglyceride lipase activity [68], inhibition of both jejunal cholesterol absorption and ileal bile acid resorption and modifying lipoprotein metabolism (decrease of low density lipoprotein). Alternatively, the high cystine content of Spirulina's C-phycocyanin protein may contribute to its hypocholesterolemic action [69].

A negative connection was found between blood cholesterol concentrations and cystine levels in dietary protein in rats fed a high cholesterol diet [70]. Spirulina has been shown to have a hypolipidemic impact because the C-phycocyanin protein inhibits pancreatic lipase activity in a dose-dependent way [71]. Controversy: The unexpected rise in TG in SP supplemented dogs may indicate that the Spirulina dose was insufficient to influence plasma TG or that the supplementation duration was insufficient for Spirulina to perform its lipid-modifying effects. According to [72], Spirulina may impact plasma lipids only under hyperlipidemic situations. Triglycerides are created in sufficient quantities in the liver to supply the entire body [73]. Their large growth might be attributed to excess weight. As a result, these lipid indicators were modified over the course of the study. Indeed, administering Spirulina caused a considerable rise in lipid levels.

The activity of AST and ALT are markers of hepatotoxicity [74]. In the current investigation, Spirulina administration resulted in a substantial drop in AST and ALT levels, showing that Spirulina may protect against liver dysfunctions [11, 39, 75].

Creatinine is a breakdown product of creatine phosphate in muscle that the body typically produces at a fairly steady rate based on muscle mass [76]. The increase in creatinine levels observed in this study could be attributed to glomerulus dysfunction, which is the structures responsible for renal filtration, whereas the increase in urea could be attributed to an increase in protein catabolism caused by the high synthesis of the enzyme arginase, which intervenes in urea production, as agreed with [11].

This was verified in additional investigations, which found a rise in total serum proteins in animals given the maximum dosage of SP. These findings are consistent with those of [11, 77], who showed a rise in the rate of total serum protein in rats fed with an aqueous extract of SP at a dosage of 75 mg/kg body wt. The increased blood protein content might be attributed to spirulina's higher protein digestibility.

Lipid peroxidation alterations are associated with MDA and oxidative damage in cells, tissues and organs. MDA levels rise as a result of free radicals activation caused by fatty acids in tissue damage. As a result, the antioxidant defense system cells are activated, and oxidative activities are decreased [78]. In the current study, the MDA value was observed to be reduced on days of Spirulina treatment as compared with the zero days. SP is expected to minimize the buildup of lipid peroxidation in organisms, which is consistent with the findings of [11, 12], who obtained comparable results in rats and rabbits, respectively.

Spirulina supplementation had a considerable impact on anti-oxidant activity, as CAT and SOD levels increased significantly in SP supplemented dogs compared to the zero day group. SP pre-treatment reduced oxidative damage by scavenging free radicals via phycocyanin, polysaccharides, and SOD, hence preventing free radical formation**.** Increased GSH, CAT, and SOD concentrations, as well as lower MDA, indicate enhanced oxidative defense in animal tissues [79, 80]. These findings are consistent with recent observations that Spirulina inclusion can reduce oxidative stress, resulting in a reduction in lipid peroxidation [11, 39, 65, 81]. According to [81], spirulina supplementation resulted in considerably greater superoxide dismutase and catalase activity in erythrocytes, as well as a rise in decreased tripeptide glutathione level in grill chickens. Spirulina's antioxidative impact is attributed to its active components, including phycocyanin, polysaccharides, α-tocopherol, and ß-carotene. These compounds have significant antioxidant properties and work directly on free radicals [65]. According to [44], phycocyanin has 20 times the antioxidant activity of vitamin C. Furthermore, Spirulina includes superoxide dismutase, which operates indirectly by slowing down the rate of oxygen radical generation processes [82].

In conclusion, Spirulina platensis supplementation dramatically enhanced dogs' growth performance, antioxidant capacity, and overall health. This was evident in increased body weight, greater white blood cell counts, and higher levels of enzymatic antioxidants, in addition to reduced levels of oxidative marker (MDA), harmful lipids and liver enzymes. Spirulina supplementation for dogs as antioxidants to protect against free radicals and cellular damage caused by any stress, to boost

development, and as an immunomodulator is worth considering. Further experiments with varied dosages of Spirulina are worthwhile in order to assess the nutritional value of Spirulina more precisely.

List of abbreviations

Spirulina Platensis (SP)

Low Density lipoprotein (LDL)

Nicotinamide adenine dinucleotide phosphate (NADPH)

Colony forming unite (CFU)

Dimethyl thiazolyl diphenyl tetrazolium (MTT)

High-performance liquid chromatography (HPLC)

Total saponin content (TSC)

Total tannin content (TTC)

Total terpenes content (TTPC)

Transform Infrared (FTIR) analysis

Alanine aminotransferase (ALT),

Aspartate aminotransferase (AST),

Blood urea nitrogen (BUN),

Creatinine (Cr)

Total cholesterol (TC)

Low density lipoprotein cholesterol (LDL-C)

Superoxide dismutase (SOD)

Glutathione peroxidase (GSH- PX)

Malondialdehyde (MDA)

Catalase (CAT)

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical approval

Our experimental design, and methodology was done according to the guidelines and regulations of the Institutional Animal Care and Use committee of the Cairo University CU-IACUC (Vet CU131020241014).

Our current study protocol and methodology is reported in accordance with ARRIVE guidelines.

Data availability

Data is provided within the manuscript file.

Author 'contributions

All authors contributed to the study's conception and design. F.S.Y. prepared the SP solution and performed the phytochemical screening, HPLC, FTIR and cytotoxicity studies. M.I.O. and M.A.E. performed the experimental research and sample withdrawal. M.A.E. and M.E.A. performed all biochemical analyses and data analyses. M.I.O., F.S.Y., and A.H.G. drafted and corrected the manuscript; MAE and MEA revised it. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

TABLE 1. Cytotoxicity for SP on WI38 cell line in lung cells as a normal cells

ID	Conc, (µg/mL)	A_{620} measurements			Mean A_{620}	SEM	Viability (%)	Toxicity (%)	IC50 ± SD
		R1	R2	R3					
WI38	--------	0.715	0.711	0.719	0.715	0.0023	100.0000	0.000	µg
	1000	0.066	0.077	0.068	0.0703	0.0033	9.836829	90.16317016	
	500	0.059	0.082	0.07	0.0703	0.0066	9.836829	90.16317016	305.2 ± 1.91
Sample 1	250	0.359	0.351	0.365	0.3583	0.0040	50.11655	49.88344988	
	125	0.715	0.717	0.712	0.7146	0.0014	99.95337	0.046620047	
	62.5	0.713	0.714	0.717	0.7146	0.0012	99.95337	0.046620047	
	31.25	0.712	0.718	0.715	0.715	0.0017	100.0000	0.000000000	

SD; standard deviation, SEM; standard error of mean.

TABLE 2. Concentrations of the components of the sample compared to the standard material.

Components	Standard		Sample	
	Conc. (µg/mL)	Area	Conc. (µg/mL)	Area
Gallic acid	20	320.66	110.05	88.22
Chlorogenic acid	50	465.45	75.81	35.29
Catechin	75	413.52	0.00	0.00
Methyl gallate	15	360.47	1.50	1.80
Coffeic acid	18	285.99	2.16	1.72
Syringic acid	17.2	320.93	14.12	13.17
Rutin	50	399.99	0.00	0.00
Ellagic acid	70	705.83	0.00	0.00
Coumaric acid	20	723.95	1.24	2.25
Vanillin	12.9	440.50	2.29	3.92
Ferulic acid	20	445.94	1.66	1.85
Naringenin	30	414.13	0.00	0.00
Rosmarinic acid	50	604.54	6.10	3.69
Daidzein	20	405.06	1.59	1.61
Querectin	20	301.82	0.00	0.00
Cinnamic acid	10	704.45	0.00	0.00
Kaempferol	20	358.24	0.00	0.00
Hesperetin	20	528.59	0.00	0.00

TABLE 3. Analysis of saponins, tannins and terpenes as components of the sample

Component	Concentration (mg/g)			Mean	SD	SEM
	Read 1	Read 2	Read 3			
Saponins	61.50	61.3	61.20	61.33	0.153	0.050
Tannins	51.1	49.2	49.50	49.93	1.021	0.333
Terpenes	45	42.0	46	44.33	2.082	0.678

SD; standard deviation, SEM; standard error of mean

TABLE 4. Effect of oral supplementation with spirulina solution on haemato-biochemical analysis in dogs.

Groups / Variables	0 day Mean ± SEM	15 days post supplementation Mean ± SEM	30 days post supplementation Mean ± SEM	45 days post supplementation Mean ± SEM
Average wt. (Kg)	3.53± 0.07^{a}	5.46± 0.08^{b}	5.73± 0.18^{b}	5.93± 0.19^{b}
Hb (g/dl)	12.10± 0.70	11.36± 0.61	10.96± 0.97	10.56± 0.19
RBCs (x10^{6} u/L)	5.67± 0.16	5.64± 0.1	5.69± 0.31	5.51± 0.17
HCT (%)	39.58± 2.11	38.44± 1.65	36.67± 3.02	37.82± 0.41
MCV (fl)	69.33± 1.84	67.66± 1.58	63.33± 1.74	69.66± 2.60
MCH (pg)	21.16± 0.64	19.96± 0.73	18.90± 0.59	19.50± 1.00
MCHC (g/dl)	30.50± 0.30	29.40± 0.35	29.73± 0.16^{b}	27.90± 0.34
Plts (x10^{3} u/L)	37.00± 7.92	94.00± 9.53	66.00± 23.27	94.66± 26.61
WBCs(x10^{3}u/L)	15.67± 0.43^{a}	20.22± 1.05^{ab}	24.01± 2.95^{b}	16.70± 0.47^{a}
Neutrophils (%)	10.50± 0.54^{a}	13.75± 0.85^{ab}	15.83± 1.74^{b}	9.97± 0.60^{a}
Eosinophils (%)	0.84± 0.18	1.38± 0.21	0.92± 0.19	0.97± 0.06
Monocytes (%)	0.70±0.13^{a}	1.40± 0.14^{b}	0.68± 0.18^{a}	0.83± 0.05^{a}
Lymphocytes (%)	3.57± 0.23^{a}	3.52± 0.19^{a}	6.51± 1.04^{b}	4.81± 0.33^{ab}
		Liver functions		
AST (U/I)	76.02± 7.4^{c}	39.07± 2.67^{b}	37.69± 3.75^{ab}	21.75±1.98^{a}
ALT (U/I)	28.99± 3.52^{b}	21.12± 1.91^{ab}	16.67± 1.42^{a}	14.54± 1.37^{a}
		Kidney functions		
BUN (mg/dl)	18.80± 0.97^{a}	17.18± 1.14^{a}	35.15± 7.24^{b}	25.88± 1.57^{ab}
Creatinine (mg/dl)	1.40± 0.14	1.28± 0.07	1.36± 0.10	1.55± 0.06
		Lipid profile		
Cholesterol(mg/dl)	316.67± 29.78	294.67± 27.18	276.33± 26.29	238.67± 16.10
Triglycerides(mg/dl)	50.60± 3.99^{a}	82.65± 6.07^{c}	72.48± 5.43^{bc}	57.93± 5.12^{ab}
LDL_C (mg/dl)	95.06± 12.55^{b}	94.33± 1.60^{b}	75.66± 1.26^{ab}	58.33± 2.12^{a}

* Means having similar lowercase letter within the same row are not significantly different at *P* value less than 0.05 level of probability

TABLE 5. Effect of oral supplementation with spirulina solution on antioxidant activity in dogs.

Variables \ Groups	0 day Mean ± SEM	15 days post supplementation Mean ± SEM	30 days post supplementation Mean ± SEM	45 days post supplementation Mean ± SEM
CAT (U/l)	202.67± 7.72[a]	402.67± 7.27[b]	905.67± 1.48[c]	930.33± 6.15[d]
SOD (U/ml)	6.06± 0.56[a]	62.00± 6.8[c]	37.00± 0.98[b]	53.00± 1.61[c]
GSH (mmol/l)	235.00± 13.87[a]	255.33± 3.35[a]	296.33± 13.97[b]	323.00± 6.06[b]
MDA (nmol/ml)	40.20± 1.36[c]	21.06± 1.75[b]	5.16± 0.30[a]	1.02± 0.10[a]

* Means having similar lowercase letter within the same row are not significantly different at *P* value less than 0.05 level of probability.

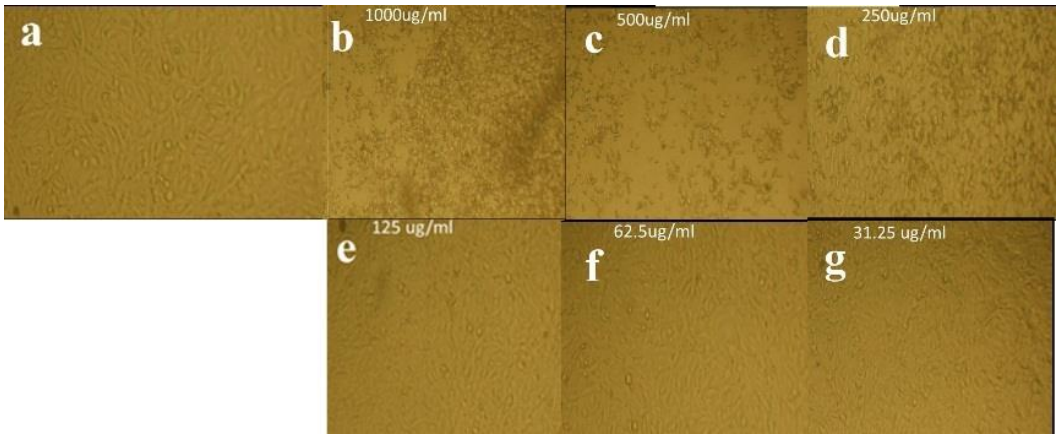

Fig. 1. (a) WI38 cell line in lung tissues as a positive control, (b) 1000 µg/mL, (c) 500 µg/mL, (d) 250 µg/mL, (e) 125 µg/mL, (f) 62.5 µg/mL, (g) 31.25 µg/mL of sample 1 for testing cytotoxicity.

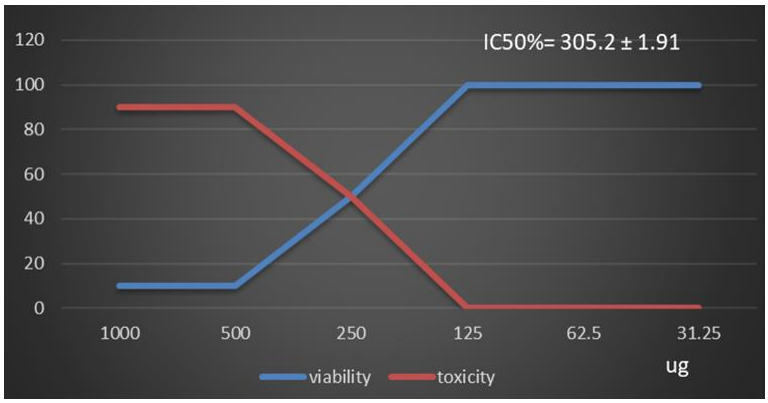

Fig. 2. Effect of sample 1 on WI38 cell line with IC_{50} of 305.2 µg/mL

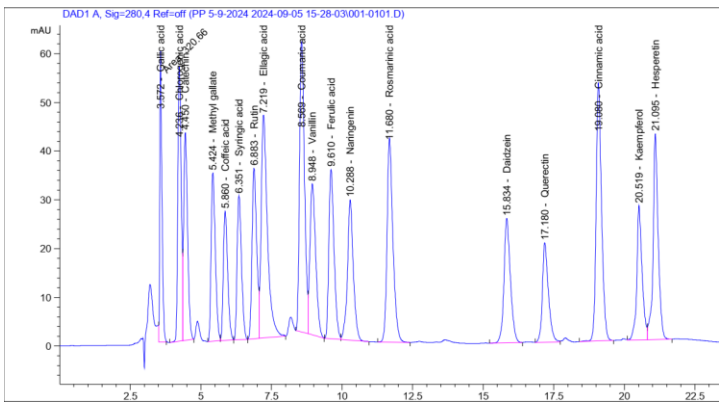

Fig. 3. HPLC analysis of standard polyphenols.

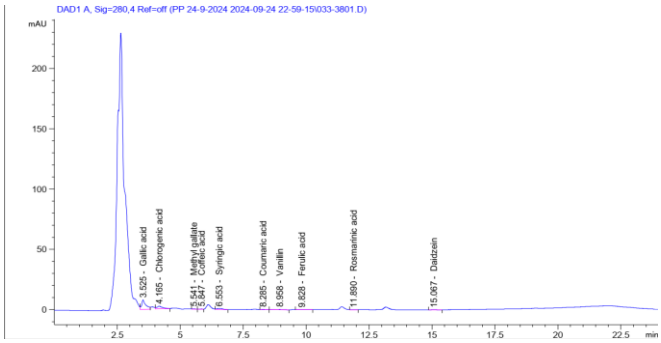

Fig. 4. HPLC analysis of sample 1.

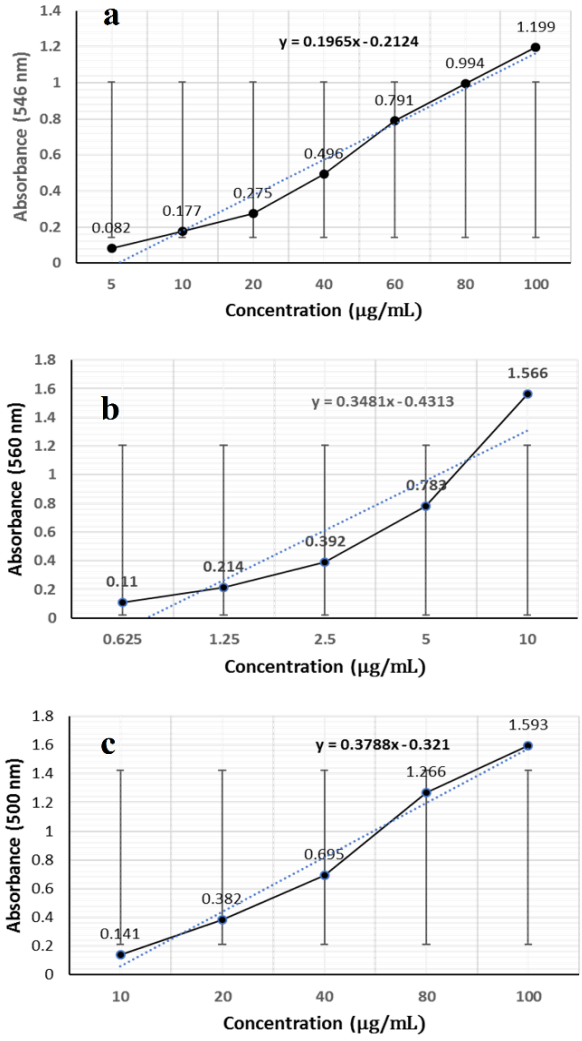

Fig. 5. Assay of different concentrations of standard substances, (a) limonene as a standard of terpenes, (b) aescin as a standard of saponins, (c) tannic acid as a standard of tannins.

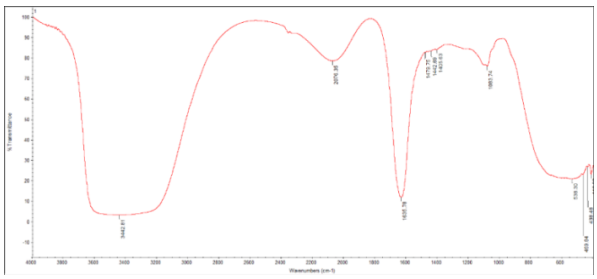

Fig. 6. FTIR analysis of the sample

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تأثير استخدام محلول السبيرولينا على النمو، والكيمياء الحيوية للدم، وتوازن الأكسدة والاختزال لدى الكلاب كمكمل غذائي فموي

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الملخص

السبيرولينا: هي طحلب دقيق أزرق مخضر، حازت على اهتمام واسع لفوائدها المحتملة في صحة الحيوان بفضل محتواها الغذائي الغني، بما في ذلك البروتينات والفيتامينات والمعادن ومضادات الأكسدة. تستكشف هذه الدراسة كيف يمكن للسبيرولينا بلاتنسيس (SP) أن تعزز صحة الحيوان. وتركز الدراسة على آثارها على وزن الجسم، والخصائص الكيميائية الحيوية للدم، وحالة مضادات الأكسدة لدى الكلاب بعد إجراء تحليل دوائي في المختبر لسمية السبيرولينا الخلوية وفعاليتها. تم تخصيص عدد خمسة عشر كلبًا متعافيًا مؤخرًا من الإصابة بالقراد، بمتوسط عمر (٣-٥ أشهر) ووزن (٣-٤ كجم)، لهذه الدراسة التجريبية، وأُعطيت ٢,٥ مل من محلول سبيرولينا بلاتنسيس بتركيز ٤٪ عن طريق الفم قبل ١٥ دقيقة من وجبة الصباح. استمرت التجربة ٤٥ يومًا، وتم الحصول على وزن الجسم وعينات الدم لكل حيوان في الأيام صفر و١٥ و٣٠ و٤٥. تم تقييم أمراض الدم والكيمياء الحيوية في المصل (الدهون، إنزيمات الكبد، وظائف الكلى وحالة المؤكسد/مضاد الأكسدة). أُجري تحليل كروماتوغرافيا السائل عالي الأداء (HPLC) لـSP ، وكشف عن وجود ١٠ جزيئات من البوليفينولات، حيث كان حمض الغاليك هو الجزيء السائد. أظهرت النتائج أن مكملات SP عززت زيادة وزن الجسم الحي، وزادت عدد كريات الدم البيضاء، ونشاط مضادات الأكسدة، وإنزيمات الكبد، ومؤشرات الأكسدة في الأيام ١٥ و٣٠ و٤٥، مع انخفاض في الدهون الثلاثية، و LDL-C بين اليومين ١٥ و٤٥، مع انخفاض غير ملحوظ في مستوى الكوليسترول مقارنةً بالأيام الصفرية) قبل إضافة مكملات SP. تشير بيانات الدراسة الحالية إلى ضرورة إضافة مكملات SP إلى وجبات الكلاب لتحسين أداء النمو، والقدرة المضادة للأكسدة، والصحة العامة.

الكلمات الدالة: الكلاب، سبيرولينا بلاتنسيس، وزن الجسم، مضادات الأكسدة، الدهون.