Original Article

Evaluation of the Side Effects of Different Doses of Spirulina on Various Organs in Rats: Biochemical, Histological and Histochemical Assessment

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ABSTRACT

Background: Spirulina is a biomass of cyanobacteria (blue-green algae) that can be consumed by humans and other animals. It is a type of microscopic algae in the shape of a perfect spiral coil. It is one of the many dietary supplements commercially available. It contains an abundant amount of essential amino acids, fatty acids, protein, vitamins, minerals, and pigments. Spirulina has many health benefits in preventing or managing hypercholesterolemia, hyperglycemia, cardiovascular diseases, diabetes, and other metabolic disease.

Aim: The principal objective of this study was to estimate the possible side effects of two doses (500 and 1000 mg/kg b. wt) of Spirulina supplementation on as the liver, kidney and testis of rats.

Materials and Methods: Adult male albino rats (Rattus norvegicus) weighing 120-140g were divided into three groups, G1: control; G2: treated with Spirulina at 500 mg/kg b. wt; and G3: treated with Spirulina at 1000 mg/kg b. wt. The administration was undertaken by gastric tube for 21 days. Biochemical analysis was done for measuring the blood levels of ALT, AST, urea, and creatinine. The liver, kidney and testis were excised, sectioned, and stained with different histological and histochemical stains for histopathological studies.

Results: Spirulina induced many histological changes in the hepatic, renal, and testicular tissues, as well as biochemical changes in a dose-dependent manner.

Conclusion: Uptake of uncontrolled supplementary doses of Spirulina may induce biochemical dysfunction and histopathological changes in some vital organs. Therefore, caution must be taken at using Spirulina as a food supplement. In fact, further biochemical and histopathological studies on its effect on other vital organs are needed for optimal dose-finding that should be considered for both efficacy and toxicity of Spirulina on the human body.

Received: 30 August 2019, **Accepted:** 28 September 2019

Key Words: Biochemistry; histochemistry; histology; rats; spirulina.

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ISSN: 1110-0559, Vol. 43, No.2

INTRODUCTION

Dietary supplements are used to compensate for the inadequately energy or nutrients (carbohydrates, lipids, proteins, vitamins, and minerals) intake to anticipate or avoid an ailment[1]. The microalgae consumption is used for the treatment of the deficiency of protein and nutrition. There are many microalgae such as Chlorella, Dunaliella, and Scenedesmus that have been utilized as food supplements[2], but Spirulina appears to be the foremost promising strain in the attempt of using unconventional sources to face the nutritional deficiencies[3,4]. Moreover, Spirulina represents one of the many dietary supplements commercially available, and it has become the focus of a great deal of food science and biochemistry research in recent years.

Spirulina is a spiral blue-green algae, belongs to cyanobacteria (microalgae) that are typically grown in water and commonly cultivated in lakes and ponds with a sufficient amount of sunlight[5]. The genus of Spirulina includes Arthrospira platensis, Arthrospira fusiformis, and Arthrospira maxima. Arthrospira platensis is the most common one. Spirulina has several benefits over plants and animals as food sources, because it can synthesize protein from inorganic nitrogen, can double its amount within hours, grow in severe conditions as pH and temperature, beside it is not season-dependent, and highly hygienic food[6]. Spirulina contains a high level of nutrient contents that matched by very few other food products, as well as high protein, unsaturated fatty acids, minerals, and vitamins content[7,8]. In addition, it has high level of antioxidants including phycocyanins, carotenoids, tocopherols and phenolic compounds[9-12]. So, it has been used as food and has essential ingredients in the natural food products and pharmaceutical components[13,14].

Spirulina platensis has many biological uses, its dietary consumption has health benefits in preventing or managing hypercholesterolemia, hyperglycemia, cardiovascular diseases, diabetes, other metabolic diseases, certain

 DOI: 10.21608/ejh.2019.16417.1162

inflammatory diseases, allergies, cancer, environmental toxicant, drug-induced toxicities, and viral infections[15-19].

In previous studies[20], the potential biological effects of Spirulina on various organs were investigated without making follow up on its adverse impact on the vital body organs. Moreover, the use of Spirulina worldwide is not only by adults, but also extends to children[21]. Therefore, the principal aim of this study was to estimate the possible side effects of Spirulina supplementation on some organs such as the liver, kidney, and testis of experimental animals.

MATERIALS AND METHODS

Chemicals

Spirulina tablets were purchased from Puritan's Pride Company, Inc. (USA). Each tablet contains 500 mg of the active ingredient. The drug was ground and administrated orally as a new suspension in 0.9% saline.

Laboratory Animals

Thirty healthy adult male Wistar albino rats (Rattus norvegicus) weighing 120-140g, were obtained from the Medical Research Center at the Faculty of Medicine, Ain Shams University (Cairo, Egypt). The rats were carefully transported to the animal care unit at the Zoology Department, Faculty of Science, Ain Shams University, two weeks before the starting of the experiment for acclimatization to laboratory conditions. The animals were reared in clean and adequately ventilated cages and bedded on fresh wood shavings. A temperature of 25°C and a 12h light/dark cycle was maintained. Free access to water and food pellets were given to the animal. All efforts were made to minimize animal suffering, and to use only the number of animals necessary to produce reliable scientific data. All animal experiments comply with the National Institutes of Health guide for the care and use of Laboratory Animals (NIH Publications No. 8023, revised 1985), and the experimental protocol was approved by the Ain Shams University Research Ethics Committee.

Experimental Design

Thirty adults male Wistar albino rats were randomly divided into three groups (n=10/group) and were assigned at random to one of the following treatments. The first group (G1) served as control (the animals ingesting distilled water). The second group (G2) was administered a low dose of Spirulina (500 mg/kg body wt); while the third group (G3) was given high dose of Spirulina (1000 mg/kg body wt). Spirulina was given daily for 21 days by gastric tube.

Sample Collection

Twenty-four hours after the last treatment, rats were decapitated. Liver, kidney, testis, and brain were excised immediately and processed for microscopic examination and stained with different histological and histochemical stains. Also, blood samples were collected from the heart, and centrifuged for biochemical assays.

Biochemical assay

The serum concentration of alanine transaminoferase (ALT), aspartate aminotransferase (AST), urea, and creatinine were determined using Biodiagnostics Reactivos GPL kits (Cairo, Egypt), according to the manufacturer's protocol, and measured by electrochemiluminescence immunoassay on a Cobas® e601 immunoassay analyzer (Roche-Hitachi Diagnostics, Mannheim, Germany).

Histological and Histochemical Procedures

Tissue samples were fixed in 10% formalin solution at room temperature for 24h. The samples were then dehydrated in ascending concentrations of ethyl alcohol, cleared in terpineol and embedded in paraffin wax. Paraffin sections (5µm thick) were stained with Hematoxylin and eosin[22] for routine histology, Periodic acid-Schiff (PAS) for glycoproteins[23], Masson trichrome for collagen[24], and Silver impregnation for brain pathology[25]. The tissues were photographed using a camera attached to a Leica DM LS2 microscope (Leica Microsystems, Wetzlar, Germany) at the Regional Center for Mycology and Biotechnology, El-Azhar University.

Statistical Analysis

Mean values and the standard error were reported as numerical data. GraphPad Prism (version 5.0, GraphPad Software, San Diego, CA, USA) was used to conduct all statistical analysis. Data were analyzed statistically using One-way ANOVA followed by post hoc multiple comparisons (Tukey's test) for a comparative study between the groups. $P<0.05$ was regarded as statistically significant.

RESULTS

Gross Morphology Observations

In the current work, all rats survived to the end of the experiment (21 days). No abnormality of behavior or morphology was detected in Spirulina treated groups. The relative weight of the studied organs did not alter compared to those of the control group (data not are shown).

Biochemical Analysis

The levels of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, and creatinine of control rats are shown in Table 1. Oral administration of rats with a low dose of Spirulina (500 mg/kg b.wt) revealed an insignificant increase in the serum levels of ALT, AST, creatinine, and urea in comparison with the control rats as shown in Figures 1 and 2, and Table 1. Whereas, the treatment with the high dose of Spirulina (1000 mg/kg b. wt) revealed highly significant increase in the liver functions, and low significant increase in the kidney functions compared to the control group (Figures 1 and 2, Table 1).

Histopathological observations

The liver

Examining liver sections of control group, stained by routine Hematoxylin and Eosin stain, revealed a typical normal hepatic architecture, with the characteristic radial cords of polyhedral hepatocytes (Figure 3a). The hepatic cells appeared with rounded, centrally located and vesicular nuclei. Blood sinusoids were running between and in parallel with the hepatic cords, and lined by flattened endothelial cells. Kupffer cells were seen on the luminal surface of the endothelial cells, within the sinusoids. (Figure 3b). The central veins of hepatic lobules of rats treated with low dose of Spirulina were slightly congested and had a few inflammatory cells (Figure 4a). Blood sinusoids with narrow spaces, bleeding portal capillaries, and cytoplasmic vacuolization of hepatocytes were also seen (Figure 4b). Some hepatocytes appeared necrotic as manifested by cytoplasmic shrinkage and nuclear pyknosis. Other hepatocytes were swollen with marked cytoplasmic eosinophilia (Figure 4c). Liver sections of rats exposed to the high dose of Spirulina revealed loss of the standard classic hepatic architecture and lobulation (Figure 5a). The severe liver injury resulted in extensive degeneration and necrosis of the hepatocytes, Pyknosis of the nuclei, cytoplasmic vacuolization of most of the liver cells, and loss of the cellular borders (Figures 5b and 5c).

Periodic acid Schiff stain (PAS) used as an indicator for the glycogen content in the hepatocytes. In control liver sections, PAS-positive granules were found in the cytoplasm of most hepatocytes as intense coarse granules displaced to one pole of the cell during the fixation period (glycogen migration phenomenon) (Figures 6a and 6b). The liver sections of rats treated with the low dose of Spirulina (G2), revealed an increase of the intensity of PAS reaction in the hepatocytes (Figures 6c and 6d). A strong PAS-reaction was illustrated in the hepatocytes of liver tissue sections of rats treated with the high dose of Spirulina (Figures 6e and 6f). In Masson's trichrome stain a few collagen fibers were seen in the wall of the central veins (Figure 7a). In low dose Spirulina-treated group, the collagen fibers were significantly increased (Figure 7b). Also, in high dose Spirulina-treated group, the fibers depositions were increased, and some blue fibers were found around the blood capillaries (Figure 7c).

The kidney

In Hematoxylin and Eosin preparations, cross-sections of the kidney of the control group display a typical histological structure of the renal cortex of healthy rats. The glomerulus is surrounded by glomerular (Bowman's) capsule which is made of simple squamous epithelium and enclosing the urinary space. The renal tubules (proximal and distal) are well developed and lined with cubical epithelial cells (Figure 8a). The kidneys of G2 and G3 groups (Figures 8b and 8c) revealed marked cellular infiltrations in the capillaries of their glomeruli. Severe inflammation and hyper-cellular infiltration were observed in the tissues treated with the high dose of Spirulina (Figure 8c).

Kidneys of rats of control group stained with periodic acid Schiff's reagent showed moderate positive stainability of their glomeruli, as well as in the basement membranes and brush borders of proximal and distal tubules. (Figure 9a). The groups treated with low and high doses of Spirulina showed an intense color of PAS reaction in the cells of renal tubules (Figures 9b and 9c). In Masson trichrome stain preparations, the collagen fibers displayed highly intense blue colourcolor especially in the basal lamina and apical regions of the different cellular structures of kidneys of Spirulina -treated groups . There was a gradual increase in the stainability and thickness of the collagen fibers with increasing the dose concentration (Figures 10b and 10c).

The Testis

Testicular tissue of the control rats that stained by Hematoxylin and Eosin showed normal structure of seminiferous tubules and interstitial tissue (Figures 11a and 11b). The seminiferous tubules are seen with spermatogonia resting on basement membrane, and other normal stages of spermatogenesis. The lumen of seminiferous tubules is filled with plenty of normal spermatozoa (Figure 11c). The testis of rats treated with the low dose of Spirulina revealed variable degrees of degenerative changes in the seminiferous tubules up to complete cellular destruction (Figures 12a and 12b). These changes were represented by necrotic cells with pyknotic nuclei and malformed spermatozoa (Figure 12c). Seminiferous tubules of rats treated with the high dose of Spirulina had much obvious destructive alterations such as marked widening of the interstitial spaces, atrophied lumen and congested blood capillaries (Figure 13a). Also, the seminiferous tubules revealed disorganized germinal epithelium with highly deformed spermatogenic cells, necrotic areas, and disappearance of almost all spermatozoa (Figure 13b).

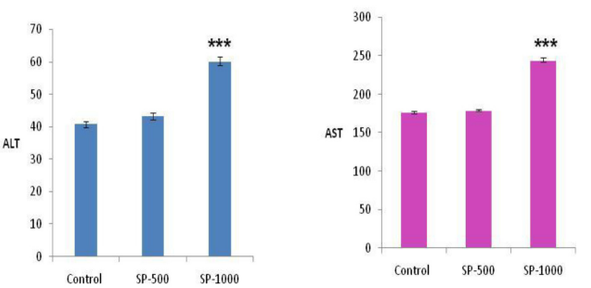

Fig. 1: Effects of Spirulina on liver biomarkers (ALT and AST) in rats. Bars represent mean ± SEM for each group (n = 10/group).
* indicates the significant difference of SP-treated groups vs. the control group *** $p<0.001$.

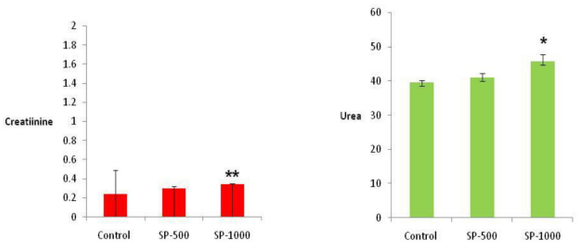

Fig. 2: Effects of Spirulina on kidney biomarkers (creatinine and urea) in rats
Bars represent mean ± SEM for each group (n = 10/group).
* indicates the significant difference of SP-treated groups vs. the control group * $p<0.05$, ** $p<0.01$.

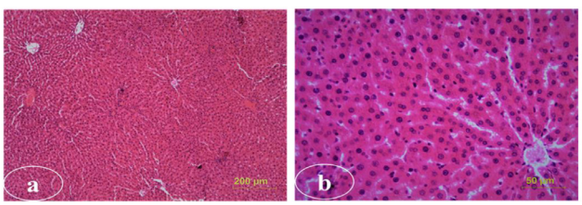

Fig. 3: Photomicrograph of liver sections stained with Hematoxylin and Eosin showing: (a) Low magnification of normal hepatic architecture of control rats. (b) High magnification of normal hepatic strands and blood sinusoids of control rats.

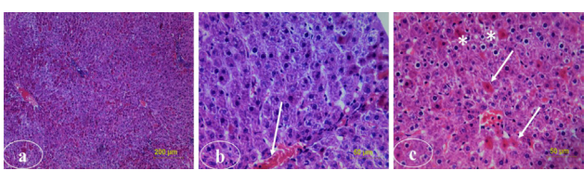

Fig. 4: Photomicrographs of liver sections stained with Hematoxylin and Eosin showing the effect of low dose of Spirulina (G2) on the liver tissues of rats. (a) Slight congestion of the central veins. (b) Cytoplasmic vacuolization of hepatocytes and bleeding in the portal capillaries (arrow). (c) Necrosis (*) of the hepatocytes and eosinophilia of some hepatocytes (arrow).

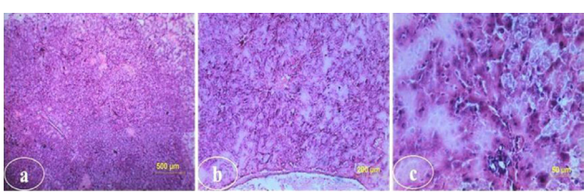

Fig. 5: Photomicrographs of liver section stained with Hematoxylin and Eosin showing the effect of high dose of Spirulina (G3) on the liver tissues of rats. (a) Disappearance of the classic hepatic lobulation. (b) Severe liver injury, the hepatocytes stained faintly and degenerated. (c) High magnification revealing sever necrosis of hepatocytes, Pyknosis of the nuclei, cytoplasmic vacuolization of most of the liver cells and loss of the cellular borders.

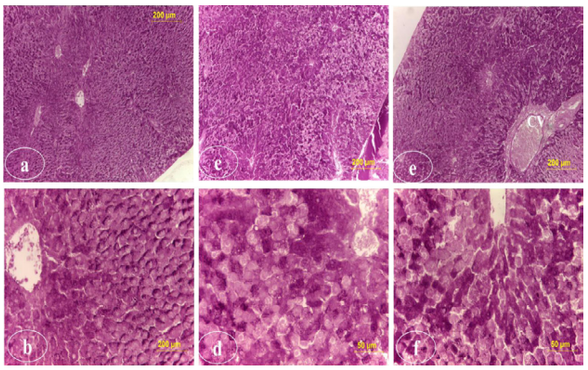

Fig. 6: Photomicrographs of liver sections stained with Periodic acid Schiff's stain (PAS) showing the distribution of carbohydrates in the hepatic tissues. (a) Liver section from control group showing normal distribution of carbohydrates. (b) Magnified hepatic cells from G1 with normal reddish carbohydrates. (c) Liver section from G2 group showing increased intensity of carbohydrate content of the hepatocytes. (d) High magnification from G2 group revealing uneven distribution of intensely-stained hepatocytes. (e) Liver section from G3 group showing high intense PAS reaction in the hepatocytes in the vicinity of dilated central veins (cv). (f) High magnification of liver section from G3 group showing uneven distribution of intense reaction of PAS in the different hepatocytes in the centrilobular area.

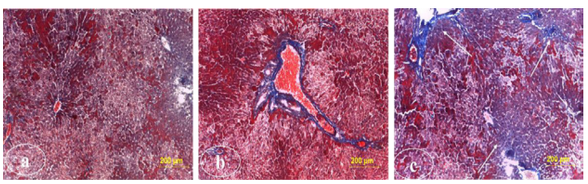

Fig. 7: Photomicrographs of liver sections stained with Masson trichrome stain showing the distribution of collagen fibers in the hepatic tissues. Collagen fibers are always stain blue. (a) Normal distribution of collagen which appears as fine blue fibers in tissues of G1. (b) Showing moderately thick collagen fibers surrounding the central vein and capillaries in the hepatic tissue of G2 group (c) Showing more intense blue collagen fibers which predominate various sites of the hepatic tissue (arrow) in liver of G3 group.

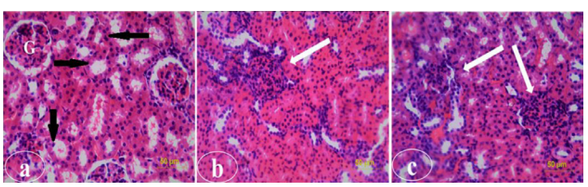

Fig. 8: Photomicrograph of kidney sections of rats stained with Hematoxylin and Eosin showing (a) Normal renal tissue architecture with glomeruli (G) and normal renal tubules (black arrow). (b) Low dose (G2 group) revealing cellular infiltrations, and enlargement of both the glomeruli (white arrow) and intertubular spaces. (c) High dose (G3-group) showing severe inflammatory infiltration and hypercellularity especially in the glomeruli.

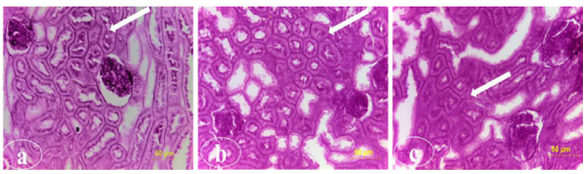

Fig. 9: Photomicrograph of kidney sections stained with Periodic acid Schiff reagents (PAS) showing: (a) Normal structure of the renal cortex. The renal tubules revealing the normal distribution of the polysaccharides in the brush borders (arrow). (b) Marked increase in the stainabilty of the cytoplasm, brush borders and basal lamina of the renal tubules of G2-group (arrow). (c) Increased staining reaction at the brush borders of the epithelial cells lining highly obliterated renal tubules in kidney section of G3-group (arrow).

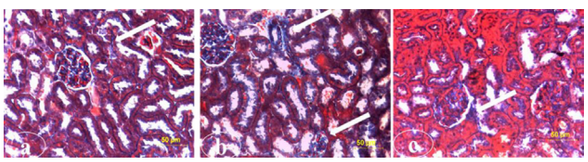

Fig. 10: Photomicrograph of kidney sections stained with Masson trichrome showing collagen fibers stained in blue (arrows). (a) Normal kidney tissues of rats of control group G1. (b) Low dose groupG2 revealing an increase in the collagen fibers stainabilty especially at the basal lamina of the epithelial cells which line the renal tubules (arrows) and inside the glomeruli. (c) High dose group revealing the presence of highly eosinophilic cells in the renal tubules, and marked increase in collagen fibers (arrow)

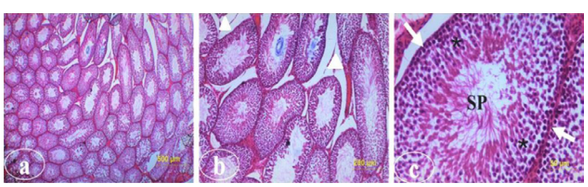

Fig. 11: Photomicrograph of cross-sections of testes of control rats. The sections stained with Hematoxylin and Eosin showing: (a) Regular arrangement of the normal seminiferous tubules. (b) Seminiferous tubules and interstitial tissue (arrow head). (c) Normal seminiferous tubule with different stages of spermatogonia (*) resting on the basement membrane (arrow). Plenty of mature normal spermatozoa (SP) in the lumen of the seminiferous tubule of control rat are observed.

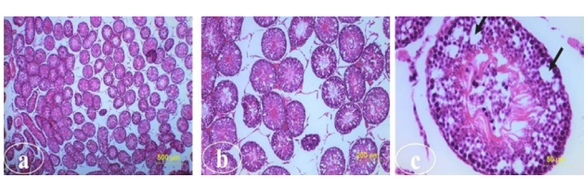

Fig. 12: Photomicrograph of cross-sections of testes of rats treated with low dose of Spirulina, and stained with Hematoxylin and Eosin, showing: (a) Irregular arrangement of seminiferous tubules. (b) Widening of interspaces in-between the tubules. (c) A seminiferous tubule with degenerated areas (arrows), necrotic cells with pyknotic nuclei and malformed sperms with their heads unusually oriented towards the lumen.

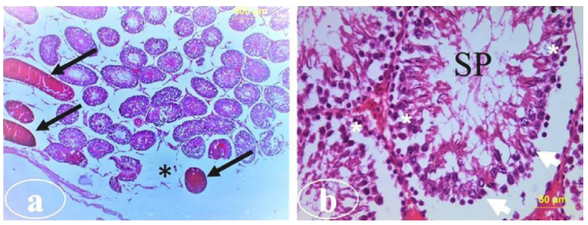

Fig. 13: Photomicrograph of cross-sections of testes of rat treated with high dose of Spirulina, stained with Hematoxylin and Eosin showing: (a) Atrophied seminiferous tubules with widened intertubular spaces (black *) and vascular congestion of some seminiferous tubules (arrow). (b) Highly disorganized and deformed seminiferous tubules with disrupted boundary (arrow), highly necrotic areas (white *) with no spermatogenic cells, and deformed spermatozoa (SP).

Table 1: Effects of Spirulina on the liver and kidney functions of rats

Parameters	Control group	SP-500 group	SP-1000 group
ALT (U/L)	40.8±0.86	43.2±1.07	60.0±1.67***
AST (U/L)	176.4±2.42	179.2±1.68	243.5±4.27***
Urea (mg/dl)	39.6±0.51	41.0±1. 3	45.75±2.08*
Creatinine (mg/dl)	0.24±0.25	0.3±0.02	0.34±0.01**

ALT: Alanine transaminase; AST: aspartate aminotransferase

Values are expressed as means ± SEM (n=10/G).

* indicates the significant difference of SP-treated groups vs. the control group * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

DISCUSSION

The blue-green algae Spirulina is one of the many dietary supplements commercially available. It has become the focus of a great deal of food science and biochemistry research in recent years[26]. It contains abundant amount of essential amino acids and fatty acids, protein, vitamins, minerals, and pigments[27-29]. As a result of its nutritional content, it serves as an alternative approach as feed, food additives, and dietary supplement[30]. Moreover, it has many pharmacological and biological activities including anticancer, antimicrobial, antiviral, Metallo-protective, antioxidant and immunostimulant effects, as well as positive effects against malnutrition, anemia, hyperlipidemia, obesity, diabetes, and inflammatory allergic reactions[15 -19]. However, information on the possible adverse effects of Spirulina is lacking. Few side effects have been reported in people taking 1g of Spirulina per day. These side effects including face flushing, headache, stomach ache, sweating, and muscle pain[10]. Therefore, the present study was designed to evaluate the possible adverse effects of two doses (500 and 1000 mg/kg b. wt) of Spirulina on various organs as liver, kidney, and testis.

In the current study, the doses of Spirulina were within the ranges of those previously used by other researchers in their studies on the impact of Spirulina on aspects as anti-inflammatory, anti-diabetes, anti-toxicity of thyroid and nervous tissues, immunostimulation, and muscle structure and shown to be effective[31-34]. In these studies, the duration of Spirulina supplementation ranged from one to eight weeks, therefore, the present experimental period extended for only 21 days (three weeks).

In the current work, all rats survived to the end of the experiment (21 days). No abnormality of behavior or morphology was detected in Spirulina treated groups. The liver function of rats treated with Spirulina, particularly the high dose, was significantly affected by the significant elevation of ALT and AST enzymes. This biochemical result agrees with the first reported case describing Spirulina-associated hepatotoxicity; in this study, significant increase in the ALT, AST, ALP, and bilirubin levels was seen after intake of Spirulina for 3-4 weeks[35]. However, other studies[36] reported that Spirulina supplementation induces insignificant difference in ALT and AST levels between the control and treated groups. As a matter of fact, ALT and AST are the key indexes measuring the level of liver cell injury. ALT is one of the most sensitive parameters for liver function tests as recommended by WHO[17,37], and is considered the most predominant enzyme in the cytosol of hepatocytes. The increase of ALT activity may be mainly due to its leakage from the hepatocyte cytosol into the bloodstream. Its elevation level is an indicator of liver cell damage and hepatic dysfunc¬tion[38-40]. AST is more in cardiac muscle than liver cells. AST includes two isoenzymes (ASTs and ASTm). In the normal serum, AST ex¬ists mainly as ASTs, and when necrosis occurs, ASTm is released from liver mitochondria, and its level in the blood serum increases[41]. These biochemical observations are most likely coincident with our histological findings, as we reported several histopathological changes in the hepatocytes of rats treated with different doses of Spirulina. These changes were dose-dependent and included; impair hepatocytes arrangement, disturbance of liver lobulation, severe degeneration, cytoplasmic vacuolization, necrosis, and pyknosis. Our observation agrees with Iwasa *et al*. (2002)[35], who reported in a case study that Spirulina intake causes liver injury; the percutaneous liver biopsy of the case showed feathery degeneration and ballooning of cells, in addition to some lymphocytic inflammation but no fat deposition or fibrosis. It is worth mentioning that cytoplasmic vacuolization may be due to the increased permeability of cell membranes, leading to an increase in intracellular water[41].

In the current investigation, the higher dose of Spirulina induced a noticeable kidney dysfunction, where the serum levels of both urea and creatinine exceeded the normal values. Urea and creatinine are the most sensitive biochemical markers employed in the diagnosis of renal damage[42]. They increase as kidney function decreases[43]. The increase in these parameters in the high dose-treated rats confirms our structural alterations. Such alterations were marked by cellular infiltrations in the glomerular capillaries in a dose-dependent manner. Similarly[44], reported in a case study that individuals using multi-ingredient products containing Spirulina develop renal failure, in addition to headache, dehydration, diarrhea, nausea, dysphasia, and oropharyngeal pain. It has been reported that Spirulina causes diarrhea and erythema after consumption of amounts corresponding to four Spirulina tablets over 3h period, in a 14-year old individual[45].

In the current study, the liver and kidney tissues treated with Spirulina revealed intense reaction with both Masson trichrome and PAS techniques compared to the control group. The severity of this reactivity was directly related to the applied dose of Spirulina. This finding is in consistent with the finding of Gargouri *et al*. (2016)[18], who mentioned that treatment of diabetic rats with Spirulina for 21 days improved glycogen level. This may be due to the induction of glycogenesis process in the liver, as reported previously in lamb[46]. In addition, Ou *et al*. (2012)[47] reported that the phycocyanin (pigment of Spirulina platensis) causes an increase in the liver glycogen content in alloxan-injured mice. The authors suggested that phycocyanin is involved in promoting the synthesis of glycogen, and enhancing the glucokinase expression that catalyzes glucose phosphorylation in pancreatic cells and hepatocytes[48].

In the current study, the testes of rats treated with a low dose of Spirulina revealed variable degrees of degenerative changes in the seminiferous tubules, including necrosis, pyknosis, unusually oriented heads of sperms towards the tubular lumen, and malformed spermatozoa, while the testes of rats treated with high dose of Spirulina showed marked widening of the interstitial spaces, atrophy of seminiferous tubules, congestion in the blood capillaries, necrosis, and disappearance of almost all spermatozoa. A particular study reported that Spirulina maxima up to high feeding levels for 13 weeks did not produce adverse effects in mice after subchronic treatment[49], except an increased relative weight of seminal vesicles, but without giving any explanation for this result. Moreover, El Arab *et al*. (2019)[50] reported that some testicular sections of irradiated rats treated with Spirulina for two months showed marked degeneration and necrosis of spermatogoneal cells lining seminiferous tubules and interstitial oedema, whereas other sections revealed normal seminiferous tubules. In addition to, a sharp disturbance in the reproductive hormones level (testosterone, FSH and LH) that may be due to the presence of certain bioactive compounds in Spirulina that might have a dysregulative effect on the reproductive hormones[51]. Some other reports are also in disagreement with our results who considered Spirulina safety in reproduction at 10%–20% feed levels[52]. In another study using acute, subchronic, and chronic levels of toxicity, Spirulina did not cause mutagenicity or teratogenicity[53]. Additionally, administration of Spirulina in rat models had a positive effect on pregnancy due to its high contents of vitamins and minerals[54-55].

These conflicting results may be related to differences in the geographical origin, harvesting period, genetic

variations, post-harvest processing conditions, the method of extraction and solvents type used[13]. Furthermore, the efficacy of Spirulina is also affected by the difference in doses, duration, administration route, frequency of supplementation. In addition to, season, animal's type, age, and sex are included[21,55].

The Mechanisms of Spirulina in the different biological effects are varied. Its mechanism as anticancer is attributed to its content of endonuclease which repair damaged DNA, as antiviral due to its calcium sulfated polysaccharide which inhibits in vitro replication of viruses, as an antimicrobial agent is due to its fatty acids (unusually high content of γ-linolenic acid), and the metal protective role of it may be due to the presence of beta-carotene, vitamins C and E, selenium, SOD and phycocyanin[29]. In fact, the adverse effects reported in the present study most likely related to the natural contamination of Spirulina with heavy metals as lead, mercury, and cadmium that may exert their effects on the body mainly by the administration of high doses of Spirulina.

CONCLUSION

Uptake of uncontrolled supplementary doses of Spirulina may induce biochemical dysfunction and histopathological changes in some vital organs. Therefore, caution must be taken at using Spirulina as a food supplement. In fact, further biochemical and histopathological studies on other vital organs are needed for optimal dose-finding that should be considered for both efficacy and toxicity of Spirulina on the human body.

CONFLICT OF INTERESTS

There are no conflict of interest

REFERENCES

1. Webb, Geoffrey P. "Nutritional supplements and conventional medicine; what should the physician know?: Symposium on 'Nutritional supplements and drug efficacy'." Proceedings of the Nutrition Society 66.4 (2007): 471-478.
2. Kay, Robert A., and Larry L. Barton. "Microalgae as food and supplement." Critical reviews in food science & nutrition 30.6 (1991): 555-573.
3. Venkataraman, L. V., *et al*. "Supplementary value of the proteins of alga Scenedesmus acutus to rice, ragi, wheat and peanut proteins [Rat diets]." Nutrition Reports International (1977).
4. Becker, E. Wolfgang. Microalgae: biotechnology and microbiology. Vol. 10. Cambridge University Press, 1994.
5. Abu-Taweel, Gasem Mohammad, *et al*. "Spirulina consumption effectively reduces anti-inflammatory and pain related infectious diseases." Journal of infection and public health (2019).
6. Kuhad, Ramesh Chander, *et al*. "Microorganisms as an alternative source of protein." Nutrition reviews 55.3 (1997): 65-75.
7. Sotiroudis, Theodore G., and Georgios T. Sotiroudis. "Health aspects of Spirulina (Arthrospira) microalga food supplement." Journal of the Serbian Chemical Society 78.3 (2013): 78-3.
8. Gupta, Ramesh C., ed. Nutraceuticals: efficacy, safety and toxicity. Academic Press, 2016.
9. Kalafati, Maria, *et al*. "Ergogenic and antioxidant effects of spirulina supplementation in humans." Med Sci Sports Exerc42.1 (2010): 142-51.
10. Mazokopakis, Elias E., *et al*. "The hypolipidaemic effects of Spirulina (Arthrospira platensis) supplementation in a Cretan population: a prospective study." Journal of the Science of Food and Agriculture 94.3 (2014): 432-437.
11. El-Sheekh, Mostafa Mohamed, Saied Mohamed Hamad, and Mahmoud Gomaa. "Protective effects of Spirulina on the liver function and hyperlipidemia of rats and human." Brazilian Archives of Biology and Technology 57.1 (2014): 77-86.
12. Serban, Maria-Corina, *et al*. "A systematic review and meta-analysis of the impact of Spirulina supplementation on plasma lipid concentrations." Clinical Nutrition 35.4 (2016): 842-851.
13. Marzieh Hosseini, Seyede, *et al*. "Spirulina paltensis: Food and function." Current Nutrition and Food Science 9.3 (2013): 189-193.
14. Czerwonka, Arkadiusz, *et al*. "Anticancer effect of the water extract of a commercial Spirulina (Arthrospira platensis) product on the human lung cancer A549 cell line." Biomedicine and Pharmacotherapy 106 (2018): 292-302.
15. El-Desoky, Gaber E., *et al*. "Improvement of mercuric chloride-induced testis injuries and sperm quality deteriorations by Spirulina platensis in rats." PLoS One 8.3 (2013): e59177.
16. Abdel-Daim, Mohamed M., *et al*. "Anti-inflammatory and immunomodulatory effects of Spirulina platensis in comparison to Dunaliella salina in acetic acid-induced rat experimental colitis." Immunopharmacology and immunotoxicology 37.2 (2015): 126-139.
17. Ibrahim, Abdelaziz E., and Mohamed Mohamed Abdel-Daim. "Modulating effects of Spirulina platensis against tilmicosin-induced cardiotoxicity in mice." Cell Journal (Yakhteh) 17.1 (2015): 137.
18. Gargouri, Manel, Christian Magné, and Abdelfattah El Feki. "Hyperglycemia, oxidative stress, liver damage and dysfunction in alloxan-induced diabetic rat are prevented by Spirulina supplementation." Nutrition research 36.11 (2016): 1255-1268.

19. Aissaoui, Ourida, *et al*. "Effect of Spirulina platensis ingestion on the abnormal biochemical and oxidative stress parameters in the pancreas and liver of alloxan-induced diabetic rats." Pharmaceutical biology 55.1 (2017): 1304-1312.
20. Hutadilok-Towatana, Nongporn, *et al*. "A subchronic toxicity study of Spirulina platensis." Food science and technology research 14.4 (2008): 351-351.
21. Sixabela, P. S. S., *et al*. "The effects of dietary supplementation with Spirulina platensis in growing rats." Asian Journal of Animal and Veterinary Advances 6.6 (2011): 609-617.
22. Carleton, Harry Montgomerie, Roger Anderson Brownsword Drury, and Eric Alfred Wallington. Carleton's histological technique. Oxford University Press, USA, 1980.
23. Hotchkiss, Rollin D. "A microchemical reaction resulting in the staining of polysaccharide structures in fixed tissue preparations." Archives of Biochemistry 16.1 (1948): 131.
24. Suvik, A., and A. W. M. Effendy. "The use of modified masson's trichrome staining in collagen evaluation in wound healing study." Mal J Vet Res 3 (2012): 39-47.
25. Fink, Robert P., and Lennart Heimer. "Two methods for selective silver impregnation of degenerating axons and their synaptic endings in the central nervous system." Brain research 4.4 (1967): 369-374.
26. Reboleira, João, *et al*. "Spirulina." Nonvitamin and Nonmineral Nutritional Supplements. Academic Press, 2019. 409-413.
27. El-Baky, Hanaa H. Abd, Farouk K. El Baz, and Gamal S. El-Baroty. "Characterization of nutraceutical compounds in blue green alga Spirulina maxima." Journal of Medicinal Plants Research 2.10 (2008): 292-300.
28. Chu, Wan-Loy, *et al*. "Protective effect of aqueous extract from Spirulina platensis against cell death induced by free radicals." BMC complementary and alternative medicine 10.1 (2010): 53.
29. Hoseini, S. M., K. Khosravi-Darani, and M. R. Mozafari. "Nutritional and medical applications of spirulina microalgae." Mini reviews in medicinal chemistry 13.8 (2013): 1231-1237.
30. Ambrosi, M. A., C. O. Reinhr, and T. E. Bertolin. "Health properties of Spirulina spp." Rev Cienc Farma Basica Apl 29.2 (2008): 109-117.
31. Parikh, Panam, Uliyar Mani, and Uma Iyer. "Role of Spirulina in the control of glycemia and lipidemia in type 2 diabetes mellitus." Journal of Medicinal Food 4.4 (2001): 193-199.
32. Han, Li-Kun, *et al*. "Isolation of pancreatic lipase activity-inhibitory component of Spirulina platensis and it reduce postprandial triacylglycerolemia." Yakugaku zasshi: Journal of the Pharmaceutical Society of Japan 126.1 (2006): 43-49.
33. Voltarelli, Fabrício A., and Maria Alice R. de Mello. "Spirulina enhanced the skeletal muscle protein in growing rats." European journal of nutrition 47.7 (2008): 393-400.
34. Banji, David, *et al*. "Investigation on the role of Spirulina platensis in ameliorating behavioural changes, thyroid dysfunction and oxidative stress in offspring of pregnant rats exposed to fluoride." Food chemistry 140.1-2 (2013): 321-331.
35. Iwasa, Motoh, *et al*. "Spirulina-associated hepatotoxicity." The American journal of gastroenterology 97.12 (2002): 3212.
36. Moreira, Lidiane Muniz, *et al*. "Spirulina as a protein source in the nutritional recovery of Wistar rats." Brazilian Archives of Biology and Technology 56.3 (2013): 447-456.
37. Mir, Mohsen, *et al*. "Toxic Effects of Cisplatin on Hepatocytes and Liver Enzymes of Rats." Anatomical Sciences Journal12.4 (2015): 171-176.
38. Pratt, D. S., *et al*. "Sleisenger and Fordtran's gastrointestinal and liver disease." Pathophysiology Diagnosis & Management, 9th edn. Philadelphia: Saunders Elsevier(2010): 1227-357.
39. Singh, A., T. K. Bhat, and O. P. Sharma. "Clinical Biochemistry of Hepatotoxicity. J Clinic Toxicol S4: 001. doi: 10.4172/2161-0495. S4-001 J Clinic Toxicol Clinical Pharmacology: Research & Trials ISSN: 2161-0495 JCT, an open access journal." vitro Systems (2011).
40. Alabdulkarim, Badriah. "Effect of camel milk on blood glucose, cholesterol, triglyceride and liver enzymes activities in female albino rats." World Appl Sci J 17.11 (2012): 1394-7.
41. Zheng, Xiao-Nan, *et al*. "Pu-erh tea powder preventive effects on cisplatin-induced liver oxidative damage in Wistar rats." Asian Pac J Cancer Prev 15.17 (2014): 7389-94.
42. Gebre, Shewit. Toxicity study of anti-ectoparasitic formulation comprising Eucalyptus globulus and Jatrophacurcas oils blended using industry based emulsifier on the histopathology of liver, kidneys and some blood parameters in mice. Diss. Addis Ababa Universty, 2018.
43. Miller O. Laboratório para o clínico. Rio de Janeiro: Atheneu, 1993. p. 493.

44. Marles, Robin J., *et al*. "United States pharmacopeia safety evaluation of Spirulina." Critical reviews in food science and nutrition 51.7 (2011): 593-604.

45. Petrus, M., *et al*. "ALLERGY Net." Allergy 65 (2009): 924-925.

46. EL-Sabagh, Mabrouk Ragab, *et al*. "Effects of Spirulina platensis algae on growth performance, antioxidative status and blood metabolites in fattening lambs." Journal of Agricultural Science 6.3 (2014): 92.

47. Ou, Yu, *et al*. "Preventive effect of phycocyanin from Spirulina platensis on alloxan-injured mice." Environmental toxicology and pharmacology 34.3 (2012): 721-726.

48. Tiedge, Markus, Tanja Richter, and Sigurd Lenzen. "Importance of cysteine residues for the stability and catalytic activity of human pancreatic beta cell glucokinase." Archives of biochemistry and biophysics 375.2 (2000): 251-260.

49. Salazar, M., *et al*. "Effect of Spirulina maxima consumption on reproduction and peri-and postnatal development in rats." Food and Chemical Toxicology 34.4 (1996): 353-359.

50. El Arab, Aliaa Ezz, Osama Ahmed Abbas, and Eman Ismail Abdel-Gawad. "Is There a Role for Spirulina Platensis against Reprotoxicity and Oxidative Stress Induced by Gamma Irradiation in Rats?." Journal of Food and Nutrition Research7.5 (2019): 377-385.

51. Fathy, Samah MM, and Ashraf MM Essa. "Influence of Spirulina platensis exudates on the endocrine and nervous systems of a mammalian model." Asian Pacific Journal of Tropical Biomedicine 5.6 (2015): 451-457.

52. Chamorro, Germán, *et al*. "Pharmacology and toxicology of Spirulina alga." Revista de investigacion clinica; organo del Hospital de Enfermedades de la Nutricion 48.5 (1996): 389-399.

53. Chamorro, Germán, *et al*. "Reproduction and peri-and postnatal evaluation of Spirulina maxima in mice." Toxicology Letters 88 (1996): 67.

54. Kapoor, Rashmi, and Usha Mehta. "Effect of supplementation of blue green alga (Spirulina) on outcome of pregnancy in rats." Plant foods for human nutrition 43.1 (1993): 29-35.

55. Salazar, M., *et al*. "Subchronic toxicity study in mice fed Spirulina maxima." Journal of ethnopharmacology 62.3 (1998): 235-241.

الملخص العربى

تقييم الآثار الجانبية لجرعات مختلفة من السبيرولينا على عدة أعضاء في الجرذان: تقييم كيميائي حيوي ونسيجى وكيميائي نسيجي

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الخلفية: السبيرولينا هي الكتلة الحيوية للبكتيريا الزرقاء (الطحالب الخضراء المزرقة) التي يمكن أن يستهلكها البشر والحيوانات الأخرى، وهى طحالب مجهرية ذات شكل لولبي مثالي. وتعتبرالسبيرولينا واحدة من العديد من المكملات الغذائية المتاحة تجارياً. يحتوي هذا النوع من المكملات الغذائيه على كمية وفيرة من الأحماض الأمينية الأساسية والأحماض الدهنية والبروتين والفيتامينات والمعادن والأصباغ. وللاسبيرولينا أيضا العديد من الفوائد الصحية في منع أو إدارة ارتفاع الكولسترول في الدم، ارتفاع السكر في الدم، وأمراض القلب والأوعية الدموية، ومرض السكري، وأمراض التمثيل الغذائي الأخرى.

الهدف: كان الهدف الرئيسي من هذه الدراسة هو تقدير الآثار الجانبية المحتملة من مكملات سبيرولينا لجرعتين (٥٠٠ و ١٠٠٠ ملغم / كغم من وزن الجسم) على الكبد والكلى والخصية الجرذان.

المواد والطرق: تم تقسيم الجرزان البيضاء الذكور البالغين (Rattus norvegicus) التي تزن ١٢٠-١٤٠ جم إلى ثلاث مجموعات: المجموعة الأولى (G١) و هي المجموعة الضابطه و المجموعة الثانية (G٢) وهى المجموعه المعامله بالسبيرولينا ٥٠٠(مجم/كجم من وزن الجسم) والمجموعة الثالثة (G٣) وهى المجموعه المعامله بالسبيرولينا (١٠٠٠ مجم/ من وزن الجسم). تم إعطاء الجرعات المحددة من الاسبيرولينا للفئران بواسطة أنبوب معدي لمدة ٢١ يومًا. تلى ذلك إجراء التقييم الكيميائي الحيوي لقياس مستويات الدم من الـ ALT و AST واليوريا والكرياتينين. كما تم استئصال الكبد والكلى والخصية وتقطيع هذه الأعضاء إلى قطاعات نسيجيه وصبغها بالصبغات المختلفه لدراسة التغيرات النسيجيه المرضيه.

النتائج: أوضحت النتائج العديد من التغيرات النسيجية في الأنسجة الكبدية والكلى والخصية الناجمه عن تناول السبيرولينا، وكذلك ظهرت تغيرات كيميائية حيوية مختلفة وذلك تبعا للجرعات المستخدمه.

الخلاصة: إن تناول جرعات غير خاضعة للرقابة من السبيرولينا قد يحفز الخلل الكيميائي الحيوي والتغيرات المرضية في بعض الأعضاء الحيوية. لذلك، يجب توخي الحذر عند استخدام السبيرولينا كمكمل غذائي. في الواقع، هناك حاجة إلى المزيد من الدراسات البيوكيميائية والنسيجيه المرضيه على الأعضاء الحيوية الأخرى من أجل العثور على الجرعة المثلى التي يجب مراعاتها من حيث فعالية وسمية السبيرولينا على جسم الإنسان.