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Novel Development of Pasta Enriched with *Spirulina platensis* Microalgae: Biochemical and Histological Parameters

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ABSTRACT

The present study aims to determine the effect of bioactive compounds (total phenolic, total flavonoids, and antioxidant activity) of Spirulina platensis powder and its pasta products on kidney and liver diseases in rats. Pasta preparation enriched in spirulina powder with a standard treatment formulation. Pasta was supplemented by adding Spirulina platensis powder at different levels (5, 10, 15, and 20 %). The results indicated that rats treated with spirulina had lower plasma levels of ALT, AST, ALP, Albumin, creatinine, and total bilirubin after the end of the experiments. However, liver glutathione (GSH) concentration was markedly decreased as compared to the control group. In conclusion, pasta enriched with spirulina improved liver and kidney functions and enhanced oxidative stress indices. Additional studies are necessary to test the application of Spirulina platensis in other contexts.

Keywords: Spirulina platensis, green pasta, Liver enzymes, in vitro model, Rats.

INTRODUCTION

Spirulina is a biomass of cyanobacteria (blue-green algae) that can be consumed by humans and other animals (El-Tantawi and Abozeid, 2019). 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 (El-Tantawi and Abozeid, 2019).

Pasta products are the most popular foods. These are normally high in starch, but low in dietary fiber, minerals, vitamins, and phenolic compounds Bianchi (Bianchi *et al.*, 2021) *et al.* (2021). To enhance the nutritional value of pasta several studies have focused on the possibility of adding functional ingredients to pasta compounds (Bianchi *et al.,* 2021). Spirulina is nature's gift as a superfood to mankind. It is a photosynthetic filamentous microalga that has emerged as a potent food supplement because of its rich micro- and macronutrient contents. The body of Spirulina is smooth and with weak cell wall that makes it easily digestible. It is a valuable source of proteins, vitamins, minerals, β- carotene, fatty acids, etc. which makes it perfect as food and fodder (Mathur, 2019). Microalgae, especially from the Spirulina genus, have an

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interesting nutritional composition, including macronutrients such as carbohydrates, lipids, proteins, vitamins, and minerals, which are essential for basic human nutrition (Mazo *et al.*, 2004). Also, to macro and micronutrients, Spirulina has other compounds with biological activities. The main bioactive compounds in cyanobacteria, such as Spirulina, are characterized by unsaturated fatty acids, amino acids, carotenoids, and phenolic compounds (Grover *et al*., 2021). These compounds are responsible for different biological activities such as antioxidant, anticarcinogenic (Tajvidi *et al.*, 2021), and neuroprotective function (Haider *et al.*, 2021). Therefore, a diet composed of products rich in some phytochemical components can reduce, for example, the risk of developing several chronic diseases, such as neurodegenerative diseases, diabetes, and cancer Liver diseases appear nowadays to be more complicated. Part of this complication may be due to our frequent contact with chemicals and other environmental pollutants. The amount of medicine consumed has increased greatly which could be dangerous to the liver (Grochowicz *et al.*, 2021).

Antioxidants are substances found in low concentrations in the body, their role is to cause retarding or inhibiting oxidative processes, and at the same time, it is susceptible to oxidation. The antioxidant body defense systems are usually endogenous, like superoxide dismutase SOD, glutathione peroxidase, and catalase); and exogenous, which come from food like flavonoids, carotenoids, vitamin E, and vitamin C; also unsaturated fatty acids UFAs (Hosseini *et al.*, 2013). Glutathione (GSH), a tripeptide particularly concentrated in the liver, is the most important tool, a reducing agent involved in the modulation of redox processes. It has also been demonstrated that GSH cannot be considered only as a mere free radical scavenger but that it takes part in the network governing the choice between survival, necrosis, and apoptosis as well as in altering the function of signal transduction and transcription factor molecules (Vairetti *et al*., 2021).

More consumers correlate eating habits and a healthy lifestyle to reduce the incidence of chronic diseases. In response to this awareness, food industries have been striving to reduce artificial additives while also developing products that provide essential nutrients and contain ingredients beneficial to health and improve physical and mental well-being (Carpentieri *et al.*, 2005).

Market and large-scale cultivation Spirulina biomass has been certified as Generally Recognized as Safe (GRAS). Because of its long history of use (FAO *et al.*, 2015), it can be commonly found and commercialized in the market in the form of dry powder, capsules, and tablets. To evaluate the antioxidant, radical scavenging, and metal-chelating activity of Spirulina algae in vitro and to evaluate their hepatoprotective effects against CCl4 in vivo. The in vitro study showed that whey protein concentrate (WPC) and Spirulina showed antioxidant, radical scavenging, and metal-chelating activities in a dose-dependent manner. The in vivo study showed that both agents succeeded in preventing liver damage induced by CCl4. This prevention was more pronounced in rats receiving the combination of WPC and Spirulina (Gad *et al.*, 2011).

The present study aims to determine the antioxidant activity of *Spirulina platensis* powder and its pasta products against Kidney and liver disease in experimental rats.

MATERIALS AND METHODS

Materials:

Raw materials:

Semolina flour is (protein 12.30%, moisture 12.0%, Carbohydrates 71.30 %, Crude Lipids 1.16 %, Fiber 0.94 % K 32.0 mg/100 g and P 50.0 mg/100g. Spirulina platensis blue alga of Cyanophyta family (protein 62.47% and moisture content 6.3%, Carbohydrates 13.53 %, Crude Lipids 6.0 %, Fiber 4.1 %, K 940 mg/ 100g and P 120 mg/100g, total carotenoids 6.50 mg/100g, Phycocyanin 185.12 mg/100 gm, Chlorophyll 1200 mg/100g, Total phenol 1350 mg Gallic acid/100g and total flavonoids 305 mg Rutin /100g) was obtained from the Algal Biotechnology Unit National Research Center, Giza, Egypt. The powder was packed in a polypropylene bag with an aluminum layer and was kept in cool, and dry conditions, away from sunlight until pasta processing. Kits were used for the determination of ALT, AST, ALP, creatinine, urea, and GSH and were purchased from Bio Diagnostic Company, Cairo-Egypt.

Animals and Experimental Design:

Forty-nine adult Sprague–Dawley females weighing 140–160 g and 5-6 weeks of age were assigned to 4 groups (n = 7) in the animal house of the Nutritional Lab., Food Sci.& Technology Dept., College of Agriculture, Al-Azhar University, Cairo, Egypt, were used in the present study. The rats were kept under normal, healthy laboratory condition conditions for 60 days; the temperature was adjusted at 25±2ºC and 12 hours light–-dark. Animals were adapted to free access to water and fed for two weeks on a basal diet before the initiation standard of the experiment according to Reeves *et al*. (1993). Standard basil diet, which is composed of 12 % casein, 10% corn oil, 4.0% salt mixture, 1% vitamins mixture, 5% fiber (bran), and 68 % corn starch as described by Ismail (2013) and Yusuf *et al*. (2014). All rates of food consumption, and weight every week for determining the body weight gain. (7 rats/group) as follows:

Group 1: Negative control rats (-ve control) fed on the standard Basel diet (nontoxic rat liver).

Group 2: Positive control rats (+ve control) fed on the standard Basel diet and after an acclimatization period Toxicity was induced by carbon tetrachloride CCl4 dissolved in olive oil (1ml/kg) weekly for four weeks ((Hsu *et al.*, 2010).

Group 3: Rats fed with pasta containing 0.0% spirulina powder. CCl4-induced toxicity, as a positive group.

Group 4: Rats nourished with pasta containing 5% spirulina powder. CCl4-induced toxicity as a positive group.

Group 5: Rats fed with pasta containing 10% spirulina powder. CCl4-induced toxicity as a positive group.

Group 6: Rats fed with pasta containing 15 % spirulina powder. CCl4-induced toxicity as a positive group.

Group 7: Rats Fed with pasta containing 20% spirulina powder. CCl4-induced toxicity as a positive group.

*All diets for induced groups of CCl4 animals except basic control diet (-ve).

Methods:

Pasta preparation enriched in spirulina powder a standard treatment formulation, including semolina flour, obtained from the pasta company (Al-Kawther, Egypt) was used. (fig.1) The microalgae powder at five different stages and levels of 0.0 (control), 5.0, 10.0, 15.0, and 20.0% by weight of the standardized and test product composition was produced. All samples were prepared in the line of Al-Kother company. Short line (form) of the plant to build enterprise it (FAVA) Italy was used. First, the control sample consisting of semolina flour, and water was produced, and then on the desired percentage, micro-algae *S. platensis* powder in 0% (control),5.0%, 10.0%, 15.0%, and 20.0 % w/w to combine raw materials added. After mixing, the batter was passed by pressure from the mold and cut to the same size as the blade that was mounted on the mold outlet. Then, the pasta was entered into pre-drying as Fradinho (Fradinho *et al.*, 2020) *et al.,* (2020). Finally, they moved into the main dryer. The drying temperature is 77 ºC. Pasta in this stage in 2 hours and 48 minutes were spent and the optimum moisture content of 12%.

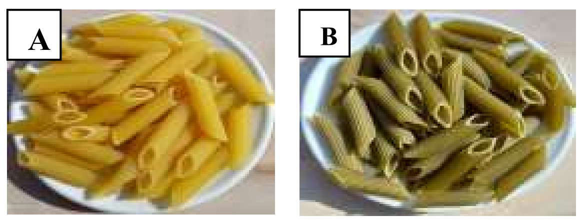

Figure 1. A :Semolina Pasta & B: Spirulina Pasta

Chemical analysis:

Moisture, crude protein, crude fat, ash, and crude fiber were determined according to the procedures of the AOAC (2002). Nitrogen-free extract of testing algae was calculated by difference. Minerals (K and P) were determined in ash solution using an Atomic Absorption Spectrometer (300VA-50-60, Hz-100-240V) UK.

Bioactive constituents:

Determination of antioxidant components:

Phenolic compounds and flavonoids in sample powder extracts were performed by HPLC analysis using the method described by Dragovic-Uzelac *et al*. (2005). Using high-performance Liquid Chromatography (HPLC) Beckman model equipped by double piston pump 126 with Fluorescence detector LC 240 (Perkin Elmer); pump for reaction (Dioxin); Derivatisering tube 10× 0.33mm; Data handling system (Software Gold); Column Supelcosil LC-18-DB, 25cm × 4.6 mm, 5µm; Injector 20µl (Beckman). The injection was carried out at wavelengths 280 nm for separation.

Determination of total phenolic content (TPC):

The total phenolic contents of the methanolic extracts of samples were determined using the Folin Ciocalteau reagent and Gallic acid was used as a standard equivalent (in mg/g) as described by Parsaei *et al*. (2013). Approximately, 500 µl of Folin–Ciocalteu reagent (Merck, Germany) were mixed with 100 µl diluted methanolic extracts. After 3 or 5 min, 400 µl 7.5% sodium carbonate was added to the mixture and allowed for 30 min at room temperature, and the absorbance was measured at 765 nm as described by (Dragovic-Uzelac *et al*., 2005).

Determination of total flavonoids:

The total flavonoids were assessed by the Aluminium chloride colorimetric method. One milliliter of the methanolic extract of samples was mixed well with 0.5 ml aluminum chloride (2%), and then 3 ml potassium acetate (5%) was added. The mixture was left for 40 min at 25 C, and the absorbance of the reaction mixture was measured at 415 nm. The calibration curve was plotted by different concentrations of rutin equivalents (in mg/100g) as described by Blasa *et al*. (2006).

Determination of antioxidant activity:

Determination of the free radical scavenging activity was done using 2,2-diphenylpicrylhydrazyl (DPPH) as a substrate according to Choi *et al.* (2016) with minor modifications. Substrate-methanol stock solution (0.004% w/v) was freshly prepared for all samples, controls, and standard curves. One mg of each sample was dissolved in 100 µl methanol solution (including negative controls); then, 3.9 ml of methanol stock solution (0.004% w/v) was added to all samples for dilution. Vigorous shaking was done at room temperature for 15 min, and then, all samples were allowed to stand in the dark for 30 min for the color development. The absorbance of all samples (including samples, blank, controls, and standard) was measured at 517 nm using a UV-Vis spectrophotometer. The percentage of DPPH was calculated according to this equation % DPPH = $[(A1–A2)/A2\times100]$; where A1 is control absorbance, and A2 is sample absorbance as described by Agustina *et al.* (2021).

Growth in rats %:

The gain in the body weights was calculated by the following equation = Final body weight (g) - initial body weight (g)/ Initial body weight (g) as indicated by Chawla (2014).

Biochemical assay:

At the end of the experimental period (60 d), blood samples were collected from the animals from the eye plexuses on ice. Each sample was collected into both heparinized tubes to obtain the plasma and put in a dry clean centrifuge glass tube to prepare serum. Blood was left for 15 min at room temperature, then the tubes were centrifuged for 15 min at 3000 rpm and the clear supernatant serum was kept frozen at -20ºC until the analysis. Liver function: Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) activities were assayed by the method of Logan *et al*. (1984) Band Ergmeyer and Harder (1986). Alkaline phosphatase (ALP) activity was measured at 405 nm by the formation of Para-nitrophenol from para-nitrophenyl phosphate as a substrate using the method of Abubakar *et al*. (2013).

Kidney function:

Creatinine and Urea were measured using the method of Fetoui *et al.* (2010). Glutathione reduced (GSH) was measured calorimetrically in erythrocytes according to the methods of El-Sayed and Rizk (2018).

Histopathological studies:

The liver was gathered from each group washed with normal saline, and kept in 10 % formalin solution for fixation, then processed routinely as described by Bancroft and Gamble (2008). The tissues were photographed using a camera attached to a Leica DM LS2 microscope (Leica Microsystems, Wetzlar, Germany) as described by Rowley *et al.* (2020).

Statistical analysis:

All data were expressed as a mean± stander error. The obtained results were subjected to the statistical analysis described by Snedecor and Cochran (1980). The sensory analysis was statistically analyzed using the standard analysis of variance one-way analysis of variance (ANOVA) using the SPSS 11-software package as described by Snedecor and Cochran (1980). Duncan's multiple range test was applied to assess significant differences between means at the 5% level of probability Duncan. Each experiment (in triplicate) was repeated, and the values are presented as the means± standard deviations.

Results and Discussion:

This work demonstrates the feeding effects of pasta enriched with spirulina algae powder against kidney and liver damage induced by CCl4 in rats (Table 1). The phycocyanin, chlorophyll, and carotenoid are an important group of pigment found in Spirulina. Carotenoids are played as lipophilic antioxidants and are responsible as an anticancer agent. The contains both of the phycocyanin, chlorophyll, and carotenoid were 180.11 and 6 mg, respectively (Liestianty,2019). Numerous studies noted that CCl4 is widely applied to induce liver damage because it is metabolized in hepatocytes by cytochrome P450, generating a highly reactive carbon-centered trichloromethyl radical, leading to initiating a chain of lipid peroxidation and thereby causing liver fibrosis (Bialy *et al*., 2019). The total phenolic, total flavonoids and total antioxidant components of Spirulina are shown in Table (1). The concentration of total phenolics was1350 mg GAE/100g); of total flavonoids, 305 mg rutin/100g) and of antioxidant activity by (DPPH %), was 93.50 % in *Spirulina platensis* powder. Similar trends were observed in the values of total phenolic compounds and DPPH by Hussein *et al*. (2021) in spirulina algae powder and pasta enriched with spirulina. These findings are conceded with the results of Schwartz and Shklar (1987). However, The Spirulina can be categorized as a superfood, because of its high nutrient content. Spirulina contains nutrients like protein, vitamins, essential fatty acids, amino acids, minerals, and phytonutrients. The high phytonutrient content of Spirulina can be considered an alternative food for vitamin supplements (Liestianty, 2019).

Table (1). Total phenolics, total flavonoids (mg/100g) content, and antioxidant activity (DPPH %) of *Spirulina platensis* powder (on a dry weight basis).

Natural antioxidant content (mg/100g)	*Spirulina platensis* powder
Total phenolics (mg GAE/100g)	1350.0 ± 1.65
Total flavonoids (mg rutin/100g)	305.0 ± 0.96
Antioxidant activity by DPPH (%)	93.50 ± 0.52

Means of three replicates ± standard error.

Due to their antioxidant properties and radical scavenging activity, much attention was gained to phenolic compounds (Yahfoufi *et al* 2018 and de Marco Castro 2019). Therefore, the phenolic content of control and fortified samples was determined using the HPLC method, and the obtained results are given in Table (2). The data showed a remarkable increase in phenolic content in supplemented pasta was recorded with increasing SP's added level and reached a maximum value at a level of 20%, which had 956.10 mg/100g. The phenolic content of SP extract was 1300.65 mg/100g in the range of previous studies carried out by Finamore *et al* (2017). The variations in obtained results between us and another investigation may be due to algae species, environmental conditions, and the origin and characteristics of used samples. However, most of the studies referred to SP as a promising source for phenolic compounds, which could be used in the food industry and pharmaceutical products. The DPPH radical scavenging activity is one of the most frequent methods used for scanning the antioxidant activity of any extract. The highest scavenging action of spirulina was also observed by Sahu *et al. (2013)* compared to other leafy vegetables. The antioxidant activity of pasta samples was analyzed by DPPH radical scavenging activity and is reported in Table (2).

Data of bioactive compounds in different treatments are depicted in Table (2). The highest levels of total phenolic, total flavonoids, and total antioxidants were observed in pa 20 and pa 15 treatments, while the lowest levels were observed in the control groups ($p < 0.05$). This result indicates that pasta fortified with spirulina powder had higher values of the bioactive compounds in a dose-dependent manner, compared to the control group. The herein findings agree with Koli *et al.* (2022) who investigated the nutritional and cooking quality of pasta enriched with spirulina powder in doses ranging between 2.5 to 15 %. Also, The obtained results agreed with Fradino *et al* (2020); who mentioned that fortification of pasta with Spirulina elevates it is antioxidant compared to the untreated sample.

Table (2). Total phenolics, total flavonoids, and antioxidant activity (DPPH %) of pasta diet fortified with different levels of *Spirulina platensis* powder (on a dry weight basis)

Samples	Total phenolics (mg GAE/100g)	Total flavonoids (mg rutin/100g)	Antioxidant activity DPPH (%)
Control (CTL -ve)	$208.40^{d} \pm 0.38$	$26.60^{d} \pm 0.22$	$15.00^{d} \pm 0.23$
Control (CTL +ve)	$200.40^{d} \pm 0.18$	$23.60^{d} \pm 0.31$	$12.00^{d} \pm 0.37$
Con. Pasta (CTL Pa 0.0 %)	$202.40^{d} \pm 0.25$	$24.60^{d} \pm 0.42$	$1600^{d} \pm 0.55$
Pasta (Pa 5%)	$656.10^{c} \pm 0.19$	$152.17^{c} \pm 0.20$	$56.56^{c} \pm 0.29$
Pasta (Pa 10%)	$775.18^{b} \pm 0.12$	$167.39^{b} \pm 0.37$	$65.15^{b} \pm 0.37$
Pasta (Pa 15%)	$893.24^{a} \pm 0.36$	$197.40^{a} \pm 0.25$	$66.16^{a} \pm 0.21$
Pasta (Pa 20%)	$956.10^{a} \pm 0.29$	$252.17^{a} \pm 0.30$	$76.56^{a} \pm 0.19$

Notes: The values are expressed as mean and standard deviation, n = 3. Means followed by different letters are significantly different ($P < 0.05$).

Changes in body weight gains and increases in liver and kidney % can be found in Table (3). Compared to the control group, the diet (Pa) caused a significant reduction ($p<0.05$) in body weight gains, on the contrary, the (Pa) diet induced an insignificant increase in body weight gains. Changes in body and liver weights and serum parameters were reported in rats poisoned with CCl_4. Treatment with CCl_4 caused a significant decrease in weight gain (%) from 27.78 to 21.27 g, in contrast, an increase in the ratio of both body weight/liver weight and body weight/kidney weight was observed when compared with the control group Table (3). In contrast, experimental animals injected with CCl_4 and fed with Spirulina pasta showed a significantly reduced ratio of liver weight to body weight and body weight/kidney weight, compared to animals injected with CCl_4. These results indicate that the Spirulina pasta effect reduces body-liver relationships and body weight/kidney weight induced by CCl_4 poisoning. This result is similar to the results of El-Sayed and Rizk (2018). The percentage of weight gain, kidney, and liver in the different treatments are depicted in Table (3). The highest weight gain percentage was observed in the negative control group followed by a 20, pa 15, and a 10 in respective order, while the lowest percentage of the same parameter was observed in positive control and CTL groups ($p < 0.05$). This result is like the results of Mohamed *et al.* (2021) and El-Sayed and Rizk (2018)who reported that Spirulina platensis prevented liver damage in CCl4-treated rats via augmentation of antioxidant defense mechanisms and inhibition of inflammatory cytokines/mediators and antiproliferative effects.

Table (3). Effect of paste diet fortified with *Spirulina platensis* powder on body weight gain and other organ weight in CCl4-induced experimental female rats.

Groups	Weight gain (%)	Liver weight (%)	Kidney weight (%)
Control (CTL -ve)	27.78^{a} ± 1.51	3.18^{d} ± 0.50	0.44^{d} ± 0.21
Control (CTL +ve)	14.55^{d} ± 1.20	4.90^{a} ± 0.11	0.62^{a} ± 0.31
Con. Pasta (CTL Pa 0.0 %)	13.94^{d} ± 1.31	4.78^{a} ± 0.40	0.63^{a} ± 0.42
Pasta (Pa 5%)	20.40^{c} ± 2.11	4.12^{b} ± 0.40	0.60^{b} ±0.36
Pasta (Pa 10%)	20.53^{c} ± 2.33	3.80^{b} ± 0.25	0.58^{b} ± 0.38
Pasta (Pa 15%)	20.70^{c} ± 1.80	3.67^{c} ± 0.16	0.56^{c} ± 0.25
Pasta (Pa 20%)	21.27^{b}± 2.01	3.55^{c} ± 0.17	0.54^{c} ± 0.32

Notes: The values are expressed as mean and standard deviation, n = 3. Means followed by different letters are significantly different ($P < 0.05$).

The nutritional and biochemical parameters of the experimental groups are presented in Table (4). It can be shown that the treatment of positive control rats with one dose of CCL4 (injected by 1 ml/kg body weight weakly) led to the development of severe hepatic injury in the rats indicated by the arising values of AST (274.0 U/L), ALT (68.2 U/L) and ALP (106.3 U/L) when compared to the control rats that have a value of AST (100.0 U/L), ALT (32.0 U/L) and ALP (64.2 U/L); respectively. When the positive control rats were treated with the Spirulina pasta diet; the tested liver enzyme activities were significantly ($p< 0.05$) decreased to lowest levels even though at all studied levels of Spirulina added (5, 10,15 and 20%). This decrease in the activities of tested liver enzymes in rat groups fed on diets containing Spirulina indicates that the pasta communicated with Spirulina is much more effective in the improvement of injury or damaged liver, which is related to their highest content of polyphenolic compounds (active antioxidants) in pasta enriched with Spirulina algae powder.

Table (4). Effect of pasta fortified with *Spirulina platensis* powder on lipid profile parameters (mg/dL) of the tested hyperlipidemic rats.

Groups	Liver Bio-markers					
	AST (U/L)	ALT (U/L)	ALP (U/L)	T. protein (g/dL)	Albumin (g/dL)	GSH (mg/dL)
Control (CTL-ve)	100.0^{c} ± 1.57	32.0^{c} ± 0.11	64.2^{c} ± 1.12	6.1^{a} ± 0.15	2.2^{d} ± 0.12	39.3^{a} ± 0.10
Control (CTL+ve)	274.0^{a} ± 2.17	68.2^{a} ± 0.33	106.3^{a} ± 1.14	5.2^{c} ± 0.40	2.2^{c} ± 0.23	21.2^{d} ± 0.15
Control Pasta (CTL Pa 0.0 %)	267.0^{a} ± 2.60	69.0^{a} ± 0.57	101.4^{a} ± 1.30	4.9^{d} ± 0.23	2.2^{d} ± 0.16	38.8^{b} ± 0.13

Pasta (Pa 5%)	$180.0^{b} \pm 1.17$	$55.0^{b} \pm 0.30$	$84.5^{b} \pm 1.24$	$5.6^{b} \pm 0.10$	$2.3^{a} \pm 0.09$	$32.1^{b} \pm 0.42$
Pasta (Pa 10%)	$157.0^{c} \pm 1.70$	$47.0^{c} \pm 0.72$	$73.0^{c} \pm 1.18$	$5.7^{b} \pm 0.15$	$2.5^{b} \pm 0.19$	$34.6^{b} \pm 0.32$
Pasta (Pa 15%)	$142.0^{c} \pm 1.30$	$40.2^{c} \pm 0.23$	$68.0^{c} \pm 1.03$	$5.8^{b} \pm 0.13$	$2.7^{b} \pm 0.11$	$35.1^{a} \pm 0.12$
Pasta (Pa 20%)	$138.0^{d} \pm 1.40$	$36.0^{d} \pm 0.47$	$63.0^{d} \pm 1.20$	$5.9^{b} \pm 0.12$	$2.8^{c} \pm 0.16$	$36.8^{a} \pm 0.12$

Notes: The values are expressed as mean and standard deviation, n = 3. Means followed by different letters are significantly different ($P < 0.05$).

The liver indices of different treatments are depicted in Table (4). The negative control group had the highest values of AST, ALT, and ALP, followed by the CTL pa and other past treatments in a dose-dependent manner, while the positive control had the lowest values of the same parameters ($p < 0.05$). The same trend was detected for GSH values. The negative control group had the highest values of total protein followed by the pasta treatments in a dose-dependent manner, while the positive control had the lowest values of the same parameter followed by the CTL pa group ($p < 0.05$). The albumin had an unclear trend. These results indicate that pasta fortified with spirulina improved liver functions and antioxidation indices.

The liver is the most sensitive organ for peroxidative damage. The induced liver fibrosis by CCl4 in rats showed marked pathological effects on hepatic stroma and parenchyma. Rats who were given spirulina powder revealed a significant decrease in elevated liver enzyme levels AST and ALT which is an important tool of the biomarker in the diagnosis of hepatic damage because they conducted with the circulation after cellular damage (Dong *et al.,* 2009). Results indicate that spirulina has a hepatoprotective effect and improved oxidative stress in hepatic cells. Any elevation of liver enzymes was caused by leakage of liver cells and disturbance and dysfunction of liver function enzymes (Bialy *et al.*, 2019). The hepatic histological changes were observed in the liver of positive control sample (CCl4) intoxicated rats.

However, the results in Table (4) cleared that the pasta containing Pa has preferable effects on the reduction of tested liver enzyme activities than that occurred when CCl4/rats were fed on a (CTL-ve) and pasta-based diet (Pa). This could emphasize that the reduction of liver enzymes tested in CCL4/rat is related to both the effects or concomitant effects of polyphenolic compounds (antioxidants) with the phytocyanin effect. These results are consistent with those of Rizk and El-Sayed (2018) who found a significant increase in AST, ALT, and ALP concentrations in rat blood serum injected with CCl4.

Albumin is the most abundant plasma protein produced by hepatocytes; its depreciation usually reflects a decrease in hepatic synthesis. This decrease is often attributed to a liver alteration in albumin synthesis. This decrease may also be due to a loss of renal function, leading to the release of albumin with urine (Sherlock, 2001). However, when the liver-intoxicated rat groups (CCl4/rats) were treated with spirulina pasta, the total serum protein content was increased with values comparable to those of the negative control rats. This was apparent in the 20% rat groups of SP-treated pasta. The results obtained are in agreement with the observation of Bialy *et al. (2019)*.

Administration of CCl4 significantly reduced GSH in rat liver, indicating the state of oxidative stress Animals treated with Pa pasta have demonstrated significant dose-proportional protection against CCl4-induced oxidative stress in rat liver. Bialy *et al*. (2019) explained the major role of oxidative stress in the progression of many chronic diseases including liver damage. Phycocyanin is one of the main components in spirulina which decreases oxidation stress in CCl4-induced lipid peroxidation animals (Thevarajah *et al*., 2022). GSH sulfhydryl is a natural antioxidant that plays a major role in protecting cells against toxic-induced oxidative stress. Our results suggest that SP has been able to prevent CCl4-induced liver damage by improving antioxidant status and reducing cellular oxidative stress. Besides, there was a relation between the hepatic tissue damage and elevation in the liver enzymes AST and ALT, due to lipid peroxidation of cell membranes that create leakage of cellular components, these harmful effects return to the toxicity of CCl4 against the liver and the body as a whole. Increment in AST and ALT (as a cytosolic creator enzyme) reflecting hepatocellular necrosis (Jarouliya *et al.,* 2012). This necrosis was improved in the hepatic tissue sections in the Spirulina-fed group to return to free radical-scavenging activity (Fig.2). After all this, we can notice that there was a proven anti-oxidant action of S. *platensis*. It was concluded that the feeding of Spirulina pasta at different levels significantly decreased the toxic effects of CCl4, by enhancing the body's defense system by scavenging the free radicals and improving the effectiveness of endogenous antioxidants. In addition to the hypolipidemic ability of *S. platensis* which indicates a protective action in the cardiovascular system. As well as improvement of hematological parameters indicates the ability to treat anemia. However, the present results are in agreement with the previous study by Hussein *et al*. (2021). For importantly, Spirulina supplementation at a high dosage of 6 g daily in NAFLD patients has strong and multiple beneficial metabolic effects and improves their health-related quality of life (Mazokopakis *et al* 2014).

Data on kidney function in different treatments are depicted in Table (5). The positive control group had the highest values of urea and creatinine, followed by the CTL pa and other past treatments in a dose-dependent manner, while the negative control group had the lowest values of the same parameters ($p < 0.05$). These results indicate that pasta fortified with spirulina improved the biological kidney functions. These kidney impacts are similar to those observed by El-Sayed and Rizk (2018). Also from Table (5), the means that Spirulina didn’t induce any elevation in Liver (AST & ALT) and kidney (urea & creatinine) functions of all studied groups appeared in normal levels compared with normal rats. However, the results obtained are in agreement with the results of Hussein et al (2022). In conclusion, the natural antioxidant microalga (*Spirulina platensis*) effectively alleviated the DZN-induced hematologic alterations and organ injuries, probably through its antioxidant and anti-inflammatory activities (Abdel-Daim *et al* 2018).

Table (5): Effect of pasta fortified with *Spirulina platensis* powder on Urea (mg/dL) and Creatinine (mg/dL) parameters of the hyperlipidemic rats.

Treatments	Urea (mg/dL)	Creatinine (mg/dL)
Control (CTL-ve)	$25.43^{c} \pm 0.54$	$0.689^{c} \pm 0.01$
Control (CTL+ve)	$38.11^{a} \pm 1.45$	$1.123^{a} \pm 0.15$
Control Pasta (CTL Pa 0.0 %)	$38.02^{a} \pm 1.21$	$1.169^{a} \pm 0.02$
Pasta (Pa 5%)	$35.22^{b} \pm 0.86$	$0.885^{b} \pm 0.03$
Pasta (Pa 10%)	$31.43^{b} \pm 1.12$	$0.794^{b} \pm 0.04$
Pasta (Pa 15%)	$27.80^{c} \pm 1.39$	$0.721^{c} \pm 0.02$
Pasta (Pa 20%)	$26.50^{c} \pm 0.86$	$0.695^{c} \pm 0.03$

Notes: The values are expressed as mean and standard deviation, n = 3. Means followed by different letters are significantly different ($P < 0.05$).

Histopathology of liver tissues is presented in Figure (2). Comparative protective effects of pasta supplemented by spirulina against subacute toxicity of carbon tetrachloride (CCl4) to rats Fig. (2 A to F). Histopathology of the liver. Fig.(2 A) represents control -ve Fig.(2 C, D) represents pasta supplemented with low ratios of Spirulina 5% treated groups and Fig.(2 E, F) represents pasta supplemented with Spirulina at high ratios of Spirulina 20% treated groups, Control group -ve shows normal hepatic histoarchitectures. Fig.(2C, D) represents the CCl4 intoxicated group: showing an extensive fatty change in peripheral hepatocytes and interlobular fibrosis and congestion. Fig.(2 E) represents the Pa + CCl4 group: showing a moderate fatty change in peripheral hepatocytes and moderate interlobular fibrosis and congestion. Fig.(2 F), represents Pa + CCl4: showing the slight greasy change in peripheral hepatocytes and slight interlobular fibrosis.

Examining liver sections of the control group, stained by routine Hematoxylin and Eosin stain, revealed a typical, normal hepatic architecture, with the characteristic radial cords of polyhedral hepatocytes Fig. (2A). 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 found on the luminal surface of endothelial cells, in the sinusoids Fig. (2B). The central veins of hepatic lobules of rats treated with a low dose of Spirulina (5%) were slightly congested and had a few inflammatory cells Fig. (2C). Blood sinusoids with narrow spaces, bleeding portal capillaries, and cytoplasmic vacuolization of hepatocytes were also seen Fig. (2D). Some hepatocytes appeared necrotic as manifested by cytoplasmic shrinkage and nuclear pyknosis. Other hepatocytes were bloated with cytoplasmic hypereosinophilia Fig. (2D). Liver sections from rats exposed to high-dose spirulina (20%) showed a loss of standard classical liver architecture and lobulation Fig. (2D). 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 cell borders Fig. (2F).

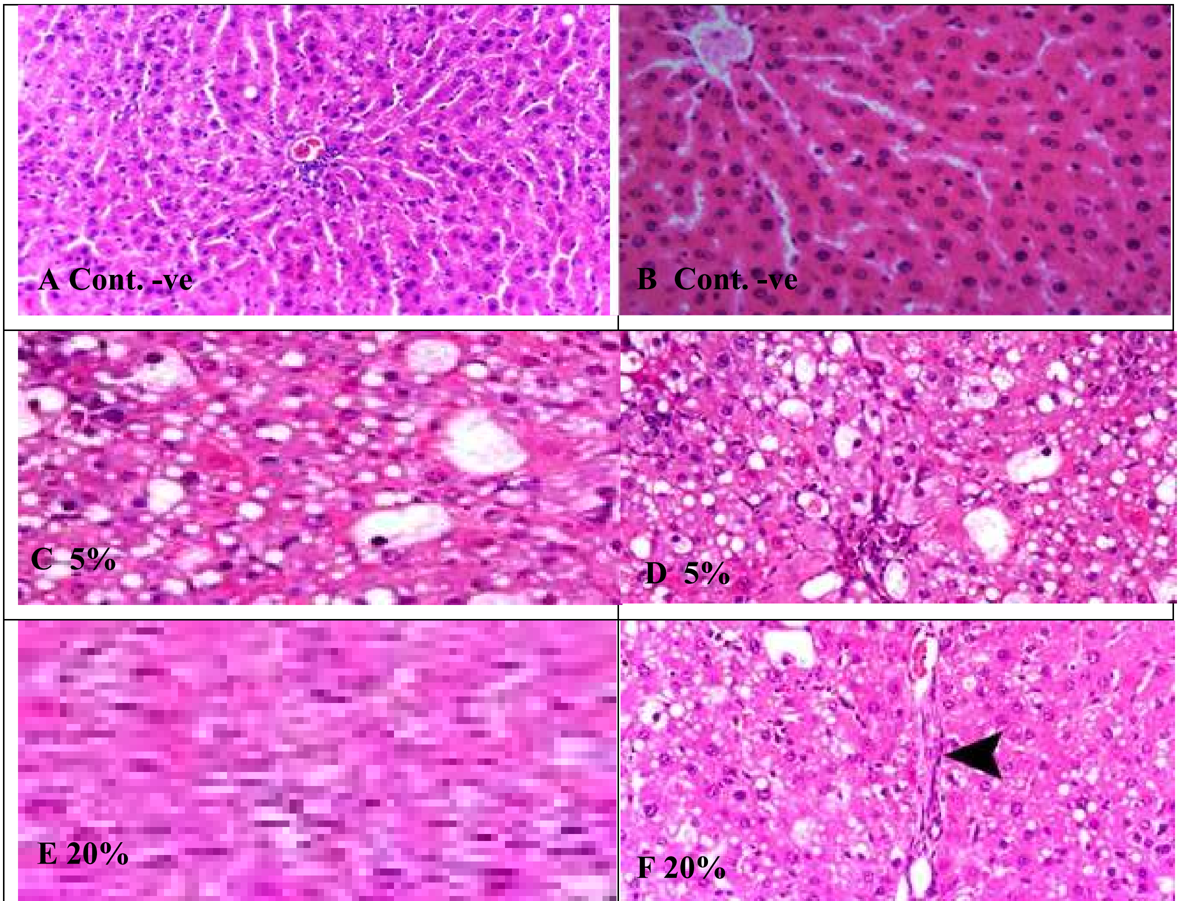

Figure (2): Hematoxylin and Eosin stain (objective×40). Histopathological observations for the protective potential of SP on CCl4 -induced hepatotoxicity in rats. (A, B -ve) Control, (C+D 5%) CCl4+SP at a low ratio and (E, F 20%) CCl4+ SP at a high ratio.

*All diet for animal groups CCL4 Induced except for control basal diet (-ve).

However, *Spirulina plantists* compounds are responsible for different biological activities such as antioxidant, anticarcinogenic (Tajvidi *et al*., 2021), and neuroprotective function (Haider *et al.*, 2021). Therefore, a diet composed of products rich in some phytochemical components can reduce, for example, the risk of developing several chronic diseases, such as neurodegenerative diseases, diabetes, and cancer Liver diseases appear nowadays to be more complicated (Lin *et al* 2016). However, From the observed results in the present study and the results of previous studies (Athane *et al* 2020 and Gutiérrez-Salmeán 2015), we can decide that Spirulina is completely safe for use as a functional food ingredient for human consumption.

Conclusions:

The present findings illustrate the dominance of bioactive compounds in the spirulina powder. Also, the spirulina powder showed a high DPPH scavenging capacity. In addition, pasta with spirulina supplementation improved the growth performance of rats. Spirulina-enriched pasta is a rich source of protein and antioxidants. The haemato-biochemical parameters were improved with increasing spirulina supplementation levels in terms of total protein, kidney, and liver function enzymes. Consequently, spirulina supplementation could be recommended to improve the nutritional quality of plant-based diets and assure healthy, and high-quality products. Further studies are needed to investigate the efficacy of a vegetarian diet supplemented with spirulina against infectious diseases and environmental pollution.

Authors Contributions:

Author Contributions: El-Sharnouby, Gamal: Writing - original draft; Investigation; Methodology; Formal analysis; Conceptualization; Validation; Visualization; Software; Data curation, Mahmoud Abughoush: Conceptualization; Investigation; Writing - review & editing; Methodology; Validation; Supervision. Imranul H. Choudhury: Writing - review & editing; Methodology; Formal analysis.

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تطوير جديد للمعكرونة المدعمة بطحالب سبيرولينا بلاتنسيس الدقيقة: المؤشرات البيوكيميائية والنسيجية

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ملخص

تهدف الدراسة الحالية إلى تحديد تأثير المركبات النشطة بيولوجيا (مجموع الفينول، إجمالي الفلافونويد، ونشاط مضادات الأكسدة) لمسحوق سبيرولينا بلاتنسيس ومنتجات المعكرونة الخاصة بها على أمراض الكلى والكبد في الجرذان. تم إعداد المعكرونة المدعمة بمسحوق سبيرولينا في تركيبة العلاج القياسية. تم استكمال المعكرونة بإضافة مسحوق سبيرولينا بلاتنسيس بمستويات مختلفة (5، 10، 15، و20٪). أشارت النتائج إلى أن الفئران المعالجة بالسبيرولينا كان لديها مستويات بلازما أقل من ALT ، AST، ALP، الألبيومين، الكرياتينين، والبيليروبين الكلي بعد انتهاء التجارب. ومع ذلك، انخفض تركيز الجلوتاثيون (GSH) في الكبد بشكل ملحوظ مقارنة بالمجموعة الضابطة. في الختام، المعكرونة المدعمة بالسبيرولينا تحسن وظائف الكبد والكلى وتعزز مؤشرات الإجهاد التأكسد. من الضروري إجراء دراسات إضافية لاختبار تطبيق سبيرولينا بلاتنسيس في سياقات أخرى.

الكلمات الدالة: سبيرولينا بلاتنسيس، المعكرونة الخضراء، إنزيمات الكبد، في النموذج المخبري، الجرذان.

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