RESEARCH PAPER

Chemical Characterization of Green Algae and Evaluation of Its Hepatoprotective Effects Against Carbon Tetrachloride-Induced Hepatotoxicity in Rats.

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ABSTRACT

Background: Carbon tetrachloride (CCl_4) induces oxidative stress and metabolic disturbances, leading to hepatic and systemic dysfunction. Green algae are a potential source of bioactive phytochemicals with antioxidant and hepatoprotective properties.Objective: This study aimed to chemically characterize green algae based on their total phenolic and flavonoid contents and evaluate their protective effects against CCl_4-induced hepatic and metabolic toxicity in rats. Materials and Methods: Thirty male rats were acclimatized for one week and randomly assigned to five groups (n = 6). The negative control received a basal diet, whereas the remaining groups were exposed to CCl_4. One group served as the CCl_4-treated control, while three groups received diets supplemented with green algae at 5%, 10%, or 15% for 28 days. Total phenolic and flavonoid contents were determined as indicators of the algae's phytochemical profile. Hepatic and renal function, antioxidant status, serum glucose, and lipid profile were assessed. Results: Green algae supplementation attenuated CCl_4-associated biochemical disturbances, improving hepatic and renal function and enhancing antioxidant status. Supplementation also favorably modulated serum glucose and lipid parameters. The protective effects were dose dependent, with the 15% supplementation level producing the most pronounced improvements. The presence of phenolic and flavonoid compounds supports the antioxidant potential of the algae and may contribute to the observed protective effects. Conclusion: Green algae supplementation mitigated CCl_4-induced hepatic and metabolic disturbances in rats, with the greatest efficacy observed at 15%. These findings highlight green algae as a promising natural source of bioactive compounds with potential hepatoprotective and antioxidant applications.

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Graphical Abstract : Protective Effects of Green Algae Against Carbon Tetrachloride-Induced Hepatic and Metabolic Toxicity in Rats: Potential Role of Phenolic and Flavonoid Compounds

Keywords: *Green algae; Phenolic compounds; Flavonoids; Carbon tetrachloride toxicity; Oxidative stress; Metabolic disturbances...*

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1. INTRODUCTION

Liver injury induced by xenobiotic chemicals represents a major focus in toxicological and pharmacological research, as it serves as a model for the oxidative and metabolic disturbances underlying many human hepatic disorders. Carbon tetrachloride (CCl_4) is one of the most widely used experimental hepatotoxins due to its consistent ability to induce oxidative stress, lipid peroxidation, disruption of antioxidant defense systems, and histopathological liver damage in animal models (Fareed et al., 2024). When metabolized in the liver by cytochrome P450 enzymes, CCl_4 generates reactive trichloromethyl (CCl_3) and trichloromethyl peroxy (CCl_3O_2) radicals, which initiate a cascade of oxidative events that impair cellular function and compromise metabolic homeostasis (Unsal et al., 2020). Oxidative stress, characterized by excessive generation of reactive oxygen species (ROS) and depletion of endogenous antioxidants such as glutathione and superoxide dismutase (SOD), plays a central role in CCl_4-mediated hepatic injury (Raj et al., 2024). These ROS promote lipid peroxidation, mitochondrial dysfunction, and inflammatory signaling, resulting in elevated serum aminotransferases and structural hepatocyte damage, hallmarks of compromised hepatic function. Therefore, interventions that enhance antioxidant capacity and reduce oxidative stress are critical strategies for mitigating CCl_4-induced liver dysfunction (Algefare et al., 2024). Green microalgae, including *Spirulina platensis* and related species, are rich in bioactive compounds such as phycobiliproteins, polyphenols, carotenoids, and essential fatty acids, many of which have demonstrated antioxidant, anti-inflammatory, and metabolic regulatory properties in vitro and in vivo (Guil & Prates, 2025). In experimental hepatotoxicity models, dietary supplementation with algae biomass or extracts has been shown to attenuate biochemical markers of liver injury, enhance the activity of endogenous antioxidant enzymes, and improve histological architecture, suggesting a protective role against oxidative damage (Altinok et al., 2020). These effects may be mediated through free radical scavenging, suppression of pro-inflammatory cytokines, and modulation of lipid metabolism.

Green microalgae, including *Spirulina platensis* and *Chlorella* species, are widely recognized for their high content of bioactive compounds, including phycobiliproteins, polyphenols, carotenoids, vitamins, and essential fatty acids. These molecules have shown potent antioxidant, anti-inflammatory, immunomodulatory, and metabolic regulatory activities in both in vitro and in vivo studies, protecting cells from oxidative damage and

supporting metabolic homeostasis (Dimopoulou et al., 2025). Phycobiliproteins, for example, scavenge ROS and reduce lipid peroxidation, while polyphenols and carotenoids enhance endogenous antioxidant defenses and modulate inflammatory signaling pathways. Essential fatty acids contribute to membrane integrity and influence lipid and glucose metabolism. Collectively, these bioactive molecules make green microalgae promising dietary supplements for mitigating chemically induced hepatic injury and improving overall physiological and metabolic status in experimental models (Ampofo & Abbey, 2022).

Despite growing evidence for the hepatoprotective potential of algae supplements, significant knowledge gaps remain regarding their effectiveness in preserving functional status and metabolic homeostasis at different dietary concentrations in CCl_4-challenged animals. Most prior studies have evaluated single doses or focused on limited biochemical endpoints, leaving uncertainties about dose-dependent responses and comprehensive effects on metabolic balance, including lipid profiles, glucose regulation, and overall physiological status. Addressing these gaps is essential to establish robust nutritional strategies that support hepatoprotection and metabolic stability under chemical stress.

Accordingly, the present study was designed to evaluate the regulatory role of graded green algae supplementation in maintaining functional status and metabolic homeostasis in rats exposed to CCl_4. We hypothesize that increasing concentrations of algae in the diet will mitigate oxidative damage, support antioxidant defenses, and improve metabolic outcomes compared with untreated CCl_4-exposed animals.

2. Materials and Methods

2.1. Materials

Green algae (Chlorophyceae): Green algae were obtained from health product and dietary supplement stores in Al-Baha City, Kingdom of Saudi Arabia (KSA).

Experimental animals: Thirty adult male albino rats of the Sprague Dawley strain, weighing 150 ± 10 g, were obtained from the Institute of Nutrition, Cairo, Egypt.

Dietary components and chemicals:

Casein, cellulose, and a blend of vitamins and minerals were purchased from Morgan Co., Cairo, Egypt.

Other chemicals, including formalin, ethanol, and EDTA, were obtained from El-Nasr Pharmaceutical Chemicals, El-Amereia, Cairo, Egypt.

2.2. Preparation of Green Algae powder.

Fresh green algae (Chlorophyceae) were initially dehydrated in a low-temperature oven at 40–50 °C until reaching constant weight. The dried biomass was then finely ground using a high-powered mechanical grinder to obtain a uniform powder. The powdered algae were subsequently stored in airtight, moisture-free containers at room temperature, away from light and heat, to maintain stability and bioactive compound integrity (Aljabri et al., 2023).

2.3. Experimental Animals and Housing

Thirty male Sprague Dawley rats were acclimatized for one week under controlled laboratory conditions: temperature 22 ± 2 °C, relative humidity 50–60%, and a 12-hour light/dark cycle. Animals had ad libitum access to standard basal diet and water during the acclimatization period. All procedures were conducted in accordance with institutional guidelines for the care and use of laboratory animals.

2.4. Diet Composition

Basal diet: The control diet was formulated according to Morsi (1992) and contained: Casein, 10%; choline chloride, 0.25%; vitamin mixture, 1%; cellulose, 5%; maize oil, 10%; salt mixture, 4%; methionine, 0.35%; corn starch, 69.5%.

Mineral supplementation: The basal diet was supplemented with the following minerals (Hegsted et al., 1941): $CaCO_3$, 600 mg; $MgSO_4 \cdot 2H_2O$, 204 mg; K_2HPO_4, 645 mg; $CaHPO_4 \cdot 2H_2O$, 150 mg; $Fe(C_6H_5O_7) \cdot 26H_2O$, 55 mg; $ZnCl_2$, 0.5 mg; $MnSO_4 \cdot 4H_2O$, 10 mg; NaCl, 334 mg; $CuSO_4 \cdot 5H_2O$, 0.06 mg; KI, 1.6 mg.

Vitamin supplementation: Vitamins were added to the basal diet according to Campbell (1963):Vitamin A, 200 IU; Vitamin K, 0.50 IU; Vitamin E, 10 IU; calcium pantothenate, 0.40 mg; thiamin, 0.50 mg; pyridoxine, 1.00 mg; vitamin D, 100 IU; folic acid, 0.02 mg; niacin, 4.00 mg; para-aminobenzoic acid, 0.02 mg; choline chloride, 200 mg; inositol, 24 mg; vitamin B_{12}, 2.00 µg.

2.5. Experimental Diet

The experimental diets consisted of the basal diet supplemented with powdered green algae at a 10% inclusion rate. Diets were freshly prepared weekly, stored at 4 °C, and provided to animals ad libitum throughout the experimental period. Table 1 summarizes the composition of the experimental diets.

Table (1): The basic and experimental diets' compositions.

Component (g)	Control (-)	Control (+)	5% green algae	10% green algae	15% green algae
Test ingredients	---	---	5	10	15
Casein	20	20	20	20	20
Corn oil	4.7	4.7	4.7	4.7	4.7
Mineral mix	3.5	3.5	3.5	3.5	3.5
Vitamin mix	1	1	1	1	1
Cellulose	5	5	5	5	5

Cholin chloride	2	2	2	2	2
Sucrose	10	10	10	10	10
Corn starch	**Up to 100**	**Up to 100**	**Up to 100**	**Up to 100**	**Up to 100**

2.6. Carbon Tetrachloride (CCl_4)

Carbon tetrachloride (CCl_4) was obtained from El-Gomhoria Company for Chemical Industries, Cairo, Egypt, as a 10% solution in liquid form. The compound was distributed in white plastic bottles (1 L) and used as a hepatotoxic agent for inducing liver injury (Passmore & Eastwood, 1986). For administration, CCl_4 was diluted with paraffin oil obtained from a local pharmacy during the induction phase.

2.7. Experimental Animals and Housing Conditions

Mature male Sprague Dawley albino rats, aged 14–16 weeks and weighing 150–160 g, were transferred from the Animal Laboratory. Animals were housed in clean plastic cages with stainless steel covers under standardized laboratory conditions. Rats were provided ad libitum access to basal diet and water via small-mouth bottles fitted with metallic tubes and plastic tubing. A 12-hour light/dark cycle was maintained. All animals were acclimated to the basal diet and housing conditions for seven days prior to the start of the study.

2.8. Induction of Liver Injury

Chronic liver injury was induced using CCl_4 as described by Jayasekhar, Mohanan et al (1997). Rats received intramuscular injections of CCl_4 in paraffin oil (50% v/v) at a dose of 2 mL/kg body weight, administered twice weekly for two consecutive weeks. Blood samples were collected via the retro-orbital route following the last injection to confirm liver injury and assess liver function.

2.9. Experimental Design and Animal Grouping

A total of 30 rats were randomly assigned to five groups, with six animals per group, as follows:

G1 (Positive Control): Normal rats fed basal diet for 28 days without any treatment.

G2 (Negative Control): Rats with CCl_4-induced liver toxicity fed basal diet for 28 days without treatment.

G3: Rats with CCl_4-induced liver toxicity fed basal diet supplemented with 5% green algae.

G4: Rats with CCl_4-induced liver toxicity fed basal diet supplemented with 10% green algae.

G5: Rats with CCl_4-induced liver toxicity fed basal diet supplemented with 15% green algae.

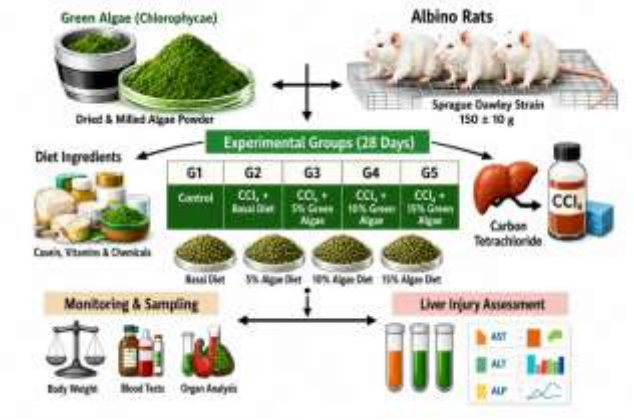

Figure 1. Experimental Methodology Overview: Effect of Green Algae on in CCl_4-Induced Rats.

2.10. Biological Evaluation

Daily feed intake was recorded throughout the 28-day experimental period. Body weight was measured weekly. Feed efficiency ratio (FER), body weight gain percentage (BWG%), and relative organ weights were calculated according to Chapman, Castillo, and Campbell (1959).

2.11. Blood Sampling and Organ Collection

At the end of the experimental period, rats were fasted for 12 hours before blood collection. Blood samples were obtained via the retro-orbital technique using specialized glass capillary tubes, allowed to coagulate for 30 minutes at 37 °C, and then centrifuged at 3000 rpm for 10 minutes to separate serum. Serum samples were aliquoted into clean polypropylene tubes and stored at −20 °C until biochemical analysis. The liver, kidney, heart, and spleen were excised, rinsed in saline, weighed, and preserved in 10% formalin for histological examination (Drury & Wallington, 1980).

2.12. Analytical Biochemistry

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities: Determined using spectrophotometric kits (BioMerieux) according to Reitman and Frankel (1957).

Alkaline phosphatase (ALP) activity: Measured colorimetrically following Roy (1970).

Serum total bilirubin: Quantified calorimetrically at 578 nm according to Doumas et al. (1973).

Total protein (TP) concentration was determined following the procedure described by Varley et al. (1980).

Serum lipid profile:

Total cholesterol measured as described by Ratliff and Hall (1973).

Triglycerides and HDL determined using enzymatic colorimetric methods (Jacobs & Van Denmark, 1960).

VLDL and LDL calculated using the method of Lee and Nieman (1996).

2.13. Statistical Analysis

Data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using analysis of variance (ANOVA) for a completely randomized design (Armitage et al., 1987). Duncan's multiple range test was used to determine significant differences among group means at $p < 0.05$.

2.14. Ethical Approval

All experimental procedures were reviewed and approved by the Research Ethics Committee of Al-Baha University (Ref. No. 46123022), Approval date: 17 April 2025.

4. RESULTS

As shown in Table 2, Green Algae (Chlorophyta) powder exhibited a total flavonoid content of 40.20 ± 1.30 µg/ml, a total phenolic content of 39.35 ± 3.3 µg/ml, and a DPPH radical scavenging activity of 15.1 ± 1.20%. These findings indicate that the powder possesses moderate antioxidant potential, likely attributed to its phenolic and flavonoid constituents, which may contribute to its free radical scavenging capacity.

Table 2: Total Phenolic and Flavonoid Contents and DPPH Radical Scavenging Activity of *Green Algae* (Chlorophyta) Powder.

Parameter	Green Algae (*Chlorophyta*) Powder
Total flavonoid(µg/ml)	40.20±1.30
Total phenolic compounds (µg/ml)	39.35±3.3
DPPH(%)	15.1±1.20

Each value in the table represents the mean ± standard deviation of three independent replicates.

4.3. Biological evaluation

Table 3 presents the effects of green algae (Chlorophyta) powder on body weight gain over 28 days in rats with CCl_4-induced liver injury. The positive control group (Control +), which received CCl_4 without any treatment, exhibited the highest body weight gain (2.86 ± 0.17 g/28 days), reflecting an abnormal response to liver injury. In comparison, the normal control group (Control −) showed a lower weight gain (1.28 ± 0.42 g/28 days).

Rats supplemented with 5% green algae demonstrated a body weight gain similar to the positive control (2.44 ± 0.21 g/28 days), while those receiving 10% green algae had the lowest gain (1.09 ± 0.12 g/28 days), comparable to the normal control. The 15% green algae group exhibited an intermediate body weight gain (1.57 ± 0.20 g/28 days).

These findings suggest that dietary supplementation with green algae can modulate body weight gain in a dose-dependent manner under conditions of CCl_4-induced hepatic stress, with the 10% supplementation showing the most effective normalization of weight gain

Table 3: Effects of Green Algae (*Chlorophyta*) Powder on body weight gain in CCl_4-Induced Rats

Groups	body weight gain. (g/28 day)
Control (-)	$1.28^{bc} \pm 0.42$
Control (+)	$2.86^{a} \pm 0.17$
5% Green Algae	$2.44^{a} \pm 0.21$
10% Green Algae	$1.09^{bc} \pm 0.12$
15% Green Algae	$1.57^{b} \pm 0.20$

Each value represents mean ± standard deviation. Mean under the same column bearing different superscript letters are different significantly ($p \leq 0.05$)

4.3.Biochemical Analysis

Table 4 illustrates the effect of different concentrations of Green Algae (Chlorophyta) powder on liver function biomarkers (AST, ALT, TP, and TB) in CCl_4-Induced Rats.

The negative control group showed normal liver function, with AST, ALT, TP, and TB values of 89.39 ± 2.21 U/L, 40.99 ± 3.51 IU/L, 7.02 ± 0.14 U/L, and 0.13 ± 0.02 U/L, respectively. In contrast, the positive control group exhibited marked liver impairment, as AST and ALT increased sharply to 329.9 ± 13.44 U/L and 299.99 ± 5.89 IU/L, respectively. At the same time, TP decreased to 4.33 ± 0.18 U/L, while TB rose to 1.52 ± 0.13 U/L, indicating significant hepatic damage.

Supplementation with Green Algae powder improved liver function in a dose-dependent manner. In the 5% group, AST and ALT levels were reduced compared with the positive control, accompanied by an increase in TP and a decrease in TB. A greater improvement was observed in the 10%

group, where liver enzyme levels declined further and protein levels increased toward normal values.

The most pronounced improvement was observed in the 15% group, where AST and ALT levels approached those of the negative control group. TP and TB values also moved closer to normal levels, suggesting a substantial protective effect of the algae powder on liver function.

Table 4: Effects of Green Algae (*Chlorophyta*) Powder on Liver Enzymes in CCl_4-Induced Rats.

Groups	Parameters			
	AST (U/L)	**ALT IU/U**	**TP (U/L)**	**TB (U/L)**
Control (-)	89.39 d±2.21	40.99e±3.51	7.02a±0.14	0.13e±0.02
Control (+)	329.9a±13.44	299.99a±5.89	4.33d±0.18	1.52a±0.13
5% Green Algae	251.97b±6.84	249.16b±4.57	5.55c±0.24	320.6+0.58^{c}
10% Green Algae	213.08c±4.95	186.53c±4.05	6.20b±0.17	0.73c±0.58
15% Green Algae	92.48d±7.11	123.54d±5.42	6.40b±0.39	0.34d±0.12

All values are expressed as mean ± standard deviation (SD). Means within the same row bearing different superscript letters indicate statistically significant differences ($P < 0.05$). AST: aspartate aminotransferase; ALT: alanine aminotransferase; TP: total protein; TB: total bilirubin.

Table 5 illustrates the effects of different concentrations of Green Algae (Chlorophyta) powder on the lipid profile of CCl_4-induced rats.

The negative control group exhibited normal lipid profile values, with total cholesterol (TC), triglycerides (TG), HDL, LDL, and VLDL recorded at 99.99 ± 4.39, 61.98 ± 4.91, 50.20 ± 1.48, 38.33 ± 5.21, and 12.41 ± 1.09 mg/dL, respectively. In contrast, the positive control group showed significant dyslipidemia, characterized by marked increases in TC, TG, LDL, and VLDL to 270.10 ± 18.1, 227.02 ± 8.10, 193.08 ± 18.26, and 45.21 ± 1.80 mg/dL, respectively, along with a reduction in HDL to 30.90 ± 2.76 mg/dL.

Supplementation with Green Algae powder resulted in a dose-dependent improvement in lipid parameters. At 5% supplementation, TC, TG, LDL, and VLDL decreased to 174.33 ± 6.02, 128.37 ± 3.39, 107.86 ± 7.78, and 25.67 ± 0.67 mg/dL, respectively, while HDL increased to 40.79 ± 1.87 mg/dL. Further improvement was observed at 10% supplementation, with TC, TG, LDL, and VLDL reduced to 146.75 ± 4.81, 116.84 ± 5.05, 79.99 ± 6.40, and 23.36 ± 1.01 mg/dL, respectively, and HDL rising to 43.40 ± 2.3 mg/dL.

The highest supplementation level (15%) produced the most pronounced improvement, with TC, TG, LDL, and VLDL reduced to 125.91 ± 9.10, 82.60 ± 9.31, 65.09 ± 9.13, and 16.52 ± 1.22 mg/dL, respectively, accompanied by an increase in HDL to 44.32 ± 2.58 mg/dL, approaching normal control values. All values are expressed as mean ± standard deviation, and means with different superscript letters indicate significant differences at $P < 0.05$.

Table 5: Effects of Green Algae (*Chlorophyta*) Powder on lipids profile **in CCl_4-Induced Rats**

Groups	Parameters				
	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
Control (-)	99.99e±4.39	61.98e±4.91	50.20a±1.48	38.33e±5.21	12.41e±1.09
Control (+)	270.10a±18.1	227.02a±8.10	30.90d±2.76	193.08a±18.26	45.21a±1.80
5% Green Algae	174.33b±6.02	128.37b±3.39	40.79c±1.87	107.86b±7.78	25.67b±0,67
10% Green Algae	146.75c±4.81	116.84c±5.05	43.40bc±2.3	79.99c±6.40	23.36c±1.01
15% Green Algae	125.91d±9.10	82.60d±9.31	44.32b±2.58	65.09d±9.13	16.52d±1.22

Values are expressed as means ± SD; means in the same raw with the different letters are significantly different ($P < 0.05$). T C; total cholesterol, T G; triglyceride; HDL; high density lipoprotein, LDL; low density lipoprotein, VLDL; very low-density lipoprotein

Table 6 shows the effects of Green Algae (Chlorophyta) powder on kidney function in CCl_4-induced rats. The negative control group maintained normal kidney function, with urea and creatinine levels of 17.11 ± 2.84 mg/dl and 0.329 ± 0.05 mg/dl, respectively. In contrast, the positive control group exhibited clear kidney impairment, as urea and creatinine levels increased markedly to 95.10 ± 1.69 mg/dl and 1.41 ± 0.21 mg/dl, respectively.

Supplementation with Green Algae powder improved kidney function in a concentration-dependent manner. The 5% group showed noticeable reductions in both urea and creatinine levels compared with the positive control group. Greater improvement was observed in the 10% group, with further decreases in both parameters. The most substantial improvement was seen in the 15% group, where urea and creatinine levels were considerably reduced and approached the values of the negative control group, indicating a protective effect of the algae powder on renal function.

Table 6: Effects of Green Algae (*Chlorophyta*) Powder on kidney functions in CCl_4-Induced Rats

Groups	Urea (mg/ dl)	Creatinine(mg/dl)
Control (-)	17.11e±2.84	0.329d±0.05
Control (+)	95.10a±1.69	1.41a±0.21
5% Green Algae	70.74b±1.53	0.91b±0.06
10% Green Algae	56.52c±3.01	0.84b±0.02
15% Green Algae	29.016d±2.18	0.64c±0.04

Values are expressed as means ± SD; means in the same raw with the different letters are significantly different (P < 0.05).

Table 7 shows how Green Algae (Chlorophyta) powder affected serum glucose levels in CCl_4-treated rats.

Rats in the negative control group had normal glucose levels (92.91 ± 11.10 mg/dl), while those in the positive control group developed marked hyperglycemia (266.35 ± 11.72 mg/dl) due to CCl_4 exposure. Feeding the rats with Green Algae powder gradually reduced glucose in a dose-dependent manner. At 5% supplementation, glucose dropped to 166.86 ± 6.41 mg/dl, at 10% it further decreased to 157.86 ± 4.81 mg/dl, and at 15% it nearly returned to normal levels (116.43 ± 7.72 mg/dl).

All values are expressed as mean ± standard deviation (SD), and differences between groups were considered statistically significant at $P < 0.05$.

Table 7: Effects of Green Algae (*Chlorophyta*) Powder on serum Glucose in CCl_4-Induced Rats

Groups	Glucose. mg/dl
Control (-)	92.91e±11.10
Control (+)	266.35a±11.72
5% Green Algae	166.86b±6.41
10% Green Algae	157.86c±4.81
15% Green Algae	116.43d±7.72

Values are expressed as means ± SD; means in the same raw with the different letters are significantly different (P < 0.05).

Table 7 presents the effects of *Green Algae* (Chlorophyta) powder on antioxidant status in CCl_4-induced ratsIn contrast, the positive control group exhibited severe oxidative stress, as indicated by a dramatic decrease in SOD (3.01 ± 1.16 U/L) and GSH (14.01 ± 2.12 U/L).

Supplementation with Green Algae powder significantly improved antioxidant defenses in a dose-dependent manner. At 5%, SOD and GSH increased to 12.87 ± 1.55 U/L and 107.88 ± 2.58 U/L, respectively. Further improvements were observed at 10%, with SOD reaching 26.47 ± 2.55 U/L and GSH 145.12 ± 2.62 U/L. The highest supplementation (15%) almost restored antioxidant levels to normal, with SOD at 34.84 ± 1.49 U/L and GSH at 172.16 ± 2.31 U/L.

Table 7: Effects of Green Algae (*Chlorophyta*) Powder on antioxidant status in CCl_4-Induced Rats

Groups	SOD(U/L)	GSH(U/L)
Control (-)	59.93a±6.98	179.90a±2.4
Control (+)	3.01e±1.16	14.01e±2.12
5% Green Algae	12.87d±1.55	107.88d±2.58
10% Green Algae	26.47c±2.55	145.12c±2.62
15% Green Algae	34.84b±1.49	172.16b±2.31

Values are expressed as means ± SD; means in the same raw with the different letters are significantly different (P < 0.05). LDH: Lactate dehydrogenase, SOD: Superoxide Dismutase, GSH: glutathione

5- DISCUSSION

The hepatoprotective effects of green algae (Chlorophyta) powder extend beyond the simple normalization of biochemical markers, encompassing modulation of oxidative stress, inflammatory responses, and cellular signaling pathways. This is consistent with established evidence that carbon tetrachloride-induced liver injury arises primarily through metabolic activation by cytochrome P450 enzymes, generating highly reactive trichloromethyl and peroxyl radicals. These reactive species initiate lipid peroxidation, disrupt membrane integrity, and trigger downstream cascades, including inflammatory signaling and apoptosis, thereby exacerbating tissue injury (Mrwad et al., 2025). One principal mechanism by which natural compounds confer hepatoprotection is the enhancement of endogenous antioxidant systems. Phenolic-rich extracts from diverse plant sources demonstrate significant antioxidant effects in CCl_4 models. For instance, administration of Carica papaya seed extract increased the activities of glutathione, superoxide dismutase, glutathione-S-transferase, and glutathione peroxidase, while reducing lipid peroxidation and pro-inflammatory markers, including nuclear factor kappa B, tumor necrosis factor alpha, and interleukin 6. Similarly, phenolic compounds from Cnestis ferruginea significantly preserved superoxide dismutase activity and cellular integrity in hepatic and renal tissues (Yin et al., 2015). These multi-component actions reflect the complex phytochemical profile of green algae, suggesting synergistic interactions among its bioactive constituents. Regulation of inflammatory signaling represents another critical aspect of hepatoprotection. Phenolic compounds frequently suppress pro-inflammatory cytokines and signaling mediators upregulated during CCl_4 toxicity. For example, oleuropein, a well-characterized olive phenolic, markedly attenuated CCl_4-induced elevations in interleukin 6 and tumor necrosis factor alpha while preserving tissue architecture and antioxidant enzyme activity (Rahmani et al., 2024). Flavonoids such as apigenin and luteolin similarly reduce oxidative stress, inflammation, and apoptosis, implicating the suppression of cytokine signaling and inflammasome activation (Han et al., 2024). These anti-inflammatory effects likely contribute substantially to the overall hepatoprotective profile of green algae beyond direct radical scavenging activity. In addition to antioxidant and anti-inflammatory actions, modulation of cellular signaling and apoptosis is increasingly recognized as central to hepatoprotection. Flavonoids upregulate the expression of cytoprotective genes while downregulating pro-apoptotic pathways. Luteolin, for example, inhibits ferroptosis, a form of programmed cell death associated with iron-dependent lipid peroxidation, by enhancing glutathione peroxidase 4 and related antioxidant machinery. Other flavonoids also regulate key transcription factors, including nuclear factor erythroid 2–related factor 2, which orchestrates endogenous antioxidant gene expression, and nuclear factor kappa B, which governs inflammatory responses (Wang et al., 2022). Renal protection observed alongside hepatic improvements in phytochemical intervention studies is not incidental; it reflects systemic reductions in oxidative stress and inflammation. Phenolic extracts preserve renal superoxide dismutase and glutathione levels and prevent elevations in urea and creatinine, likely through attenuation of systemic reactive oxygen species and inflammatory mediators (Almundarij et al., 2021). These findings support the concept that green algae bioactives exert protective effects throughout the organism, not solely within hepatic tissue. While the 2,2-diphenyl-1-picrylhydrazyl assay provides a preliminary measure of free radical scavenging, it does not fully capture the complexity of in vivo antioxidant interactions, which involve enzyme modulation, signaling pathways, and repair mechanisms. Comprehensive assessment using markers of oxidative stress such as malondialdehyde and 4-hydroxynonenal, evaluation of transcription factor activity, and detailed histopathological examination is necessary to elucidate the underlying mechanisms. Additionally, investigations of gene expression related to antioxidant responses, inflammatory mediators, and regulators of programmed cell death, including B-cell lymphoma 2 and Bcl-2-associated X protein, will provide deeper insights into the molecular basis of hepatoprotection conferred by green algae (Mohamed et al., 2021). Collectively, evidence from diverse phytochemical studies indicates that the protective effects of natural antioxidants against CCl_4-induced liver damage involve multifaceted mechanisms: enhancement of endogenous antioxidant systems, suppression of inflammatory signaling, stabilization of cellular membranes, inhibition of programmed cell death pathways, and modulation of metabolic networks. These integrated actions likely account for the systemic benefits observed with green algae supplementation and align with contemporary understanding of how dietary phenolics mitigate oxidative stress-related pathologies.

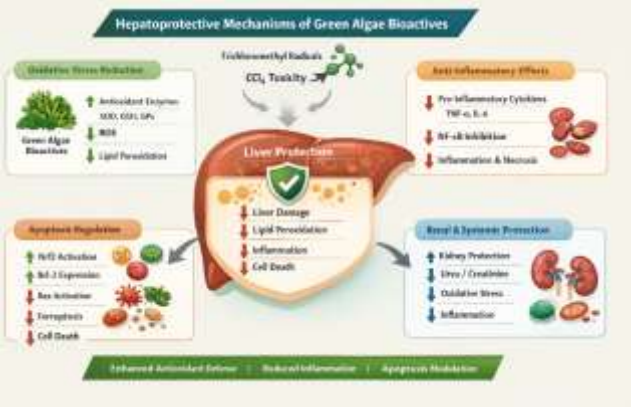

Figure 2. Hepatoprotective mechanisms of green algae bioactive compounds, illustrating antioxidant, anti-inflammatory, apoptosis-regulating, and systemic protective effects.

6- Conclusion

In conclusion, dietary supplementation with green algae (Chlorophyta) powder demonstrated significant protective effects against CCl_4-induced hepatic and renal damage, as well as metabolic disturbances, in a dose-dependent manner. The observed improvements in antioxidant status, liver enzymes, lipid profile, and glucose levels suggest that the bioactive compounds in the algae particularly flavonoids and phenolics play a central role in mitigating oxidative stress and stabilizing cellular function. The 15% supplementation consistently yielded the most pronounced benefits, highlighting the potential of green algae as a natural, safe, and effective nutraceutical for supporting liver and kidney health. These findings provide a strong basis for further studies to explore the molecular mechanisms underlying its protective effects and its potential applications in human nutrition and therapeutic strategies.

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