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Research Article

Nano-*Spirulina platensis* Outperforms Ethanol Extract and ZnO Nanoparticles in Modulating Lipid Metabolism, Liver and Cardiac Function, and Oxidative Stress in Hyperlipidemic Rats

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FMM conducted the experiments, analysed data and wrote the manuscript. HAS supervised the study and revised the manuscript. Both authors approved the final version.

Keywords
Hyperlipidemia, Rats, Nano-Spirulina platensis, ZnO nanoparticles, Oxidative stress, Cardiac biomarkers

Abstract | Hyperlipidemia is a widely found metabolic disorder characterized by the dysregulation of lipid homeostasis and the elevation of oxidative stress levels, which causes serious liver damage and cardiovascular complications. The present study evaluated and compared the therapeutic efficacy of both ethanolic extract and nano-formulation of Spirulina platensis as well as zinc oxide nanoparticles (ZnO-NPs) in ameliorating hyperlipidemia-induced alterations in albino rats. Forty male albino rats were divided into five groups: normal control (C), high-fat diet-induced hyperlipidemic group (T1), ethanolic Spirulina-treated group (T2, 100 mg/kg), nano-Spirulina-treated group (T3, 100 mg/kg) and ZnO-NPs-treated group (T4, 10 mg/kg). All interventions were followed up for 30 days. The parameters evaluated were body weight, lipid profile, hepatic enzyme activities, oxidative stress markers, and cardiac biomarkers. Results revealed that animals in the group T1 had the highest values of body weight gain, total cholesterol, triglycerides, LDL, liver enzymes AST and ALT, MDA and cardiac injury markers troponin I, CK-MB, LDH, with the lowest level of HDL and antioxidant enzymes. T3 attenuated these alterations to the greatest degree with the least weight gain and biochemical defects, the highest HDL, GSH, SOD, and CAT. Moreover, the hepatoprotective, antioxidative, and cardioprotective effects of the nano-formulated Spirulina platensis (T3) were stronger than ethanolic extract and ZnO-NPs and could offer another natural approach for controlling diet-induced hyperlipidemia and its consequences.

Novelty Statement | This study provides the first direct comparison between Nano-Spirulina platensis, ethanol extract, and ZnO nanoparticles in treating hyperlipidemia. Nano-Spirulina showed the most effective results in improving lipid profile and reducing oxidative stress. These findings suggest its potential as a novel therapeutic approach.

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Introduction

Hyperlipidemia is an alarming public health issue and is strongly linked to cardiovascular diseases, liver impairment, and metabolic syndromes. It is marked by high levels of total cholesterol (TC), triglycerides (TG), low-density lipoproteins (LDL), and very low-density lipoproteins (VLDL), with the possibility of low high-density lipoproteins (HDL) levels. Chronic administration of a high-fat diet (HFD) is also one of the most prevalent triggers of lipid imbalance and oxidative stress in mammals, which can lead to lipid peroxidation, systemic inflammation, and damage to multiple organs (Al-Rubaei *et al.*, 2017; Munshi *et al.*, 2014).

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Recently, attention has been directed towards natural bioactive compounds that can counter lipid abnormalities. Among them, Spirulina platensis, a blue-green alga (Cyanobacteria), has attracted special attention due to its very promising nutritional and medicinal potential. It contains several antioxidants, vitamins (B-complex, C, E), minerals (iron, magnesium, selenium), essential amino acids, and bioactive pigments like phycocyanin and β-carotene. Reports indicate Spirulina has antioxidant, antiinflammatory, hypolipidemic, immunostimulatory, and hepatoprotective properties (Deng and Chow, 2010; Ku *et al*., 2015).

Of course, the inclusion of Spirulina in nano-formulations to enhance its bioactivity has also been suggested. With large surface area and bioavailability, Nanoparticles encourage efficient cellular absorption and targeted delivery. Specialized nanomaterials such as Zinc Oxide Nanoparticles (ZnO-NPs) have shown potential therapeutic effects due to the antioxidative impacts and modulating effects on enzyme activity. Still, comparative studies with natural products like Spirulina remain scarce (Djearamane *et al*., 2018).

This study aims to evaluate and compare the physiological and biochemical effects of ethanolic *Spirulina* extract, nano-formulated *Spirulina*, and zinc oxide nanoparticles in a high-fat diet-induced hyperlipidemia rat model. Parameters examined include body weight, lipid profile, liver function enzymes, oxidative stress markers, and cardiac indicators. The goal is to identify which treatment offers the most protective and therapeutic benefit, potentially supporting the integration of nano-bioactive agents in managing lipid disorders.

Materials and Methods

Study design and animal model

The experiment was carried out using the animal house of the Biology Department, College of Education, University of Al-Qadisiyah. Forty adult male albino rats (10–12 weeks, 150–200 g) were obtained from the Embryo and Infertility Research Center, University of Baghdad. All animals were acclimatized in plastic cages (50 × 35 × 15 cm), raised under controlled environmental conditions (temperature: 23–25 °C; light/dark cycle: 12 h light/12 h dark), and fed a standard diet with free access to water. The experiment was conducted from October 15, 2024, to January 15, 2025.

Experimental groups

The animals used in this study were randomly assigned to five groups (n = 8 in each):

C (Negative Control): Standard chow and oral normal saline.

T1 (Positive Control): Cholesterol HFD 60 days

T2: Fed HFD + orally administered 100 mg/kg/day ethanolic Spirulina platensis extract for 30 days.

T3: Fed HFD + 100 mg/kg/day nano-formulated Spirulina platensis extract for 30 days.

T4: Fed HFD+10mg/kg/day ZnO-NPs for 30 days.

Preparation of high-fat diet (HFD)

Commercial chow powder was used to prepare an HFD by mixing with 30% melted animal fat, according to the method of Altunkaynak (2005). The blend was homogenized and air-dried at room temperature until moisture was completely evaporated.

Preparation of spirulina extract

Spirulina platensis powder (total Weight: 80 g, origin: USA) was liposolubilized in 800 mL of 70% ethanol (v/v) and stirred under 400 rpm for 72 h at 45 °C (solution filtered, concentrated by rotary evaporator, and dried in an oven below 45 °C) (the dry extract was stored in tightly closed containers at 4 °C).

Spirulina extract nano-formulation

The nano-formulation was prepared as per Kolekar *et al*. (2011). An equal volume (50mL) of ethanolic Spirulina extract was mixed with 50mL of a ZnO-NP solution (1g in 50% ethanol) in a magnetic stirrer at room temperature for 2 h. The resulting mixture was incubated for 18 h at 37°C in an orbital shaker and then at 40°C in the absence of the shaker for 24 h. The precipitate was collected via centrifugation (5000 rpm, 20 min), washed with deionized water, dried at 40 °C, and crushed into powder.

Nanoparticle characterization

FTIR analysis: Fourier transform infrared spectroscopy (FTIR) was conducted over the range of 4000–400 cm^{-1} on a spectrometer to observe the functional groups on the surface of the synthesized nanoparticles. Samples were prepared as pellets with potassium bromide (KBr) and analyzed to identify characteristic absorption bands associated with chemical bonding and surface interactions (Socrates, 2001).

XRD analysis

X-ray diffraction (XRD) analysis was performed on a diffractometer (Cu-Kα radiation, λ= 1.5406 Å, 40 kV, 30 mA). The crystalline structure, phase composition, and average crystallite size of the nanoparticles were obtained through an X-ray diffraction (XRD) scan within a 2θ range of 10°–80°, utilizing the Debye Scherrer equation (Cullity and Stock, 2001).

Dose selection

For dose selection, Ethanolic and nanoSpirulina extracts: 100mg/kg/placebo/day (Al-Gabri *et al*., 2021) and ZnO-NPs: 10mg/kg/day (Djearamane *et al*., 2018) was used to study the effects on animals in this study.

Blood and tissue collection

At the end of the treatment period, rats were anesthetized with ketamine/xylazine (1:3 ratio). Blood was collected through cardiac puncture and centrifuged to obtain serum. Serum samples were preserved at –20°C.

Biochemical analysis

Blood samples were taken via cardiac puncture after 30 days of treatment while under ketamine/xylazine anesthesia (1:3 ratio). The blood was allowed to clot at room temperature for 30 min and then centrifuged at 3000 rpm for 20 min. The serum was then maintained at –20°C until biochemical analysis.

Lipid profile

Total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) were determined in serum by commercial enzymatic colorimetric kits (BioSystems, USA and China). VLDL concentration was estimated using the following formula:

VLDL = TG/5 (Assuming triglyceride values expressed in mg/dL)

These were carried out according to established enzymatic protocols (Roeschlau *et al.*, 1974).

Liver function enzymes

The levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured using kits from BioSystems (USA) using the method of Reitman and Frankel (1957). The unit of enzyme activity was expressed as U/L.

Oxidative stress biomarkers

Malondialdehyde (MDA), A marker of lipid peroxidation (thiobarbituric acid reactive substances (TBARS) assay modified from Guidet and Shah (1989). Absorbance was measured at 532nm, with concentrations calculated using an extinction coefficient of 1.56×10^5 M^{-1} cm^{-1}. Glutathione (GSH) was determined by reaction with 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), as per Beutler *et al.* (1963) and absorbance was recorded at 412nm. Superoxide Dismutase (SOD) was measured using the SolarBio kits (China) according to the inhibition of superoxide-induced reduction of nitroblue tetrazolium (NBT) as described previously by Marklund and Marklund (1974). Catalase (CAT) was detected based on method described by Aebi (1984), it was measured at 240 nm using SolarBio assay kits.

Cardiac biomarkers

Troponin-I was assessed by ELISA-based kit (ABO, Switzerland), indicating myocardial injury.

Lactate Dehydrogenase (LDH) activity was measured by standard enzymatic kits (ABO, Switzerland) based on the conversion of lactate into pyruvate. Creatine Kinase-MB (CK-MB) was measured by immuno-inhibition method (ABO, Switzerland), a sensitive cardiac marker.

Weight gain assessment

After dose administration, weight gain assessment was determined as follows:

Weight Gain (g) = Final Weight – Initial Weight

Statistical analysis

All the results were expressed as mean ± SD and data were statistically analyzed by one-way ANOVA with LSD post hoc test, and $p < 0.05$ was taken as statistically significant.

Results

FTIR and XRD analyses

FTIR analysis: FTIR spectra showed that the ethanolic Spirulina extract exhibited prominent bands at 3404.77, 3285.53, 2929.15, 1634.71, 1533.79, 1452.92, 1052.21, and 479.6 cm^{-1}, which were assigned to O–H stretching, amine groups, and carboxylic acid functionalities. ZnO NPs showed peaks corresponding to the presence of hydroxyl groups and Zn–O bonds at 3414.63, 1630.44, 1392.56, 1042.36, 834.09, 704.05, 549.08, and 478.12 cm^{-1}. Frequencies corresponding to interactions between Spirulina constituents and ZnO nanoparticles in the new functional groups are newly formed Nano-Spirulina formulation at 3385.80; 2970.24; 1646.90; 1522.12; 1397.16; 1074.06; 762.09 and 445.97 cm^{-1}. Such spectral variations show that chemical modifications and effective incorporation of Spirulina bioactives to the nanoparticle matrix occurred (Figures 1, 2, and 3).

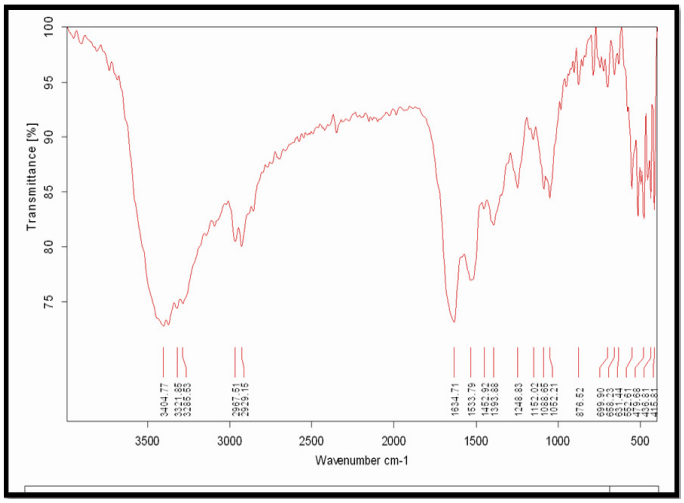

Figure 1: Fourier transform infrared (FTIR) spectrum of the ethanolic extract of *Spirulina platensis*.

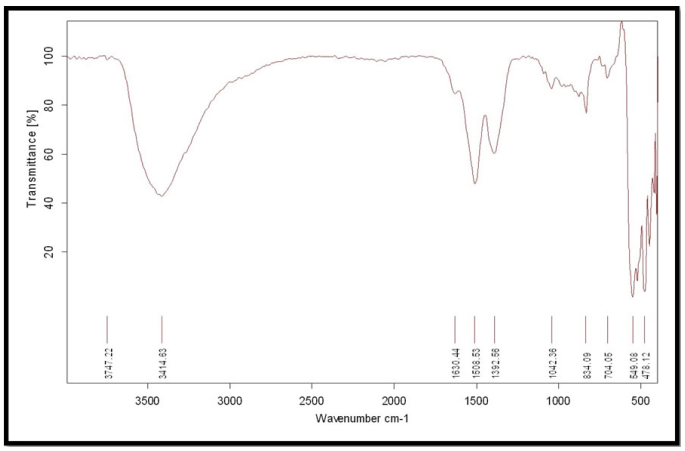

Figure 2: Fourier transform infrared (FTIR) analysis of the zinc oxide nanoparticles (ZnO-NPs).

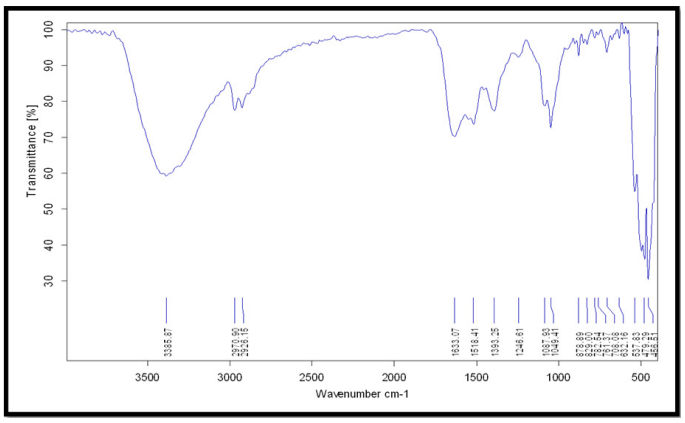

Figure 3: Fourier transform infrared (FTIR) analysis of the nano-loaded ethanolic extract of *Spirulina platensis*.

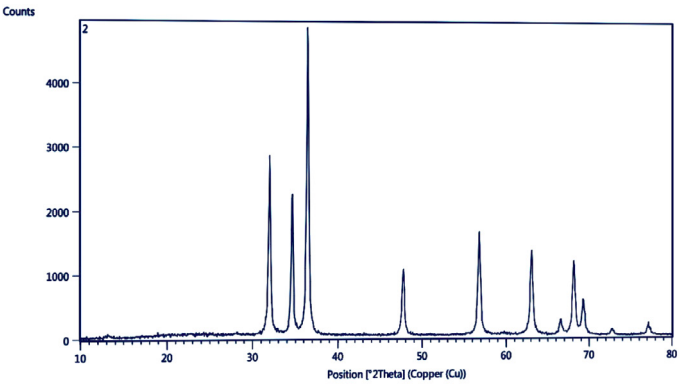

Figure 4: X-ray diffraction (XRD) result of the nano-loaded ethanolic extract of *Spirulina platensis*.

XRD analysis: As shown in Figure 4, the XRD pattern of the nano-Spirulina formulation exhibits several sharp peaks in the 20 value between 10°–80°, indicating its crystalline nature. The presence of these reflections agrees well with the nanoscale particle size and phase purity (Figure 4), suggesting adequate crystallinity and structural integrity of the formulation.

Body weight gain

Table 1 shows the effects of ethanolic and nano-formulated Spirulina platensis extracts on body weight gain in high-fat diet-induced hyperlipidemia in rats. As depicted in Table 1, the group under treatment with HFD (T1) displayed a significantly higher weight gain (208.5 ± 14.05 g) than C (97.25 ± 7.55 g), confirming the successful induction of hyperlipidemia. In contrast, groups T2 and T3 (ethanolic and nano-Spirulina extract) showed a significant decrease in weight gain (52.5 ± 15.63 g and 30.25 ± 3.01 g, respectively) compared to the T0 group. The most important weight control was demonstrated by T3, indicating the higher efficacy of the nano-formulated extract. The weight gain was significantly lower in T4 (79 ± 22.64 g), who were administered ZnO-NPs, but the effect was lesser than in T2 and T3.

Table 1: Effect of different treatments on body weight gain in experimental rat groups (Mean ± SD).

Group	Initial weight (g)	Final weight (g)	Weight gain (g)
C	259±3.18 ^{A}	356.25±4.71 ^{B}	97.25±7.55 ^{B}
T1	240±17.19 ^{A}	448.5±3.86 ^{A}	208.5±14.05 ^{A}
T2	250.25±10.11 ^{A}	302.75±20.29 ^{D}	52.5±15.63 ^{D}
T3	264±6.79 ^{A}	294.25±6.10 ^{E}	30.25±3.01 ^{E}
T4	249.25±5.39 ^{A}	328.25±19.01 ^{C}	79 ± 22.64 ^{C}
LSD	15.214	16.254	11.241

Note: Different letters indicate statistically significant differences p≤0.05

Table 2: Effect of different treatments on serum cholesterol and triglyceride levels in experimental rat groups (Mean ± SD).

Group	Cholesterol (mg/dL)	Triglycerides (mg/dL)
C	70.94±4.32^{d}	61.52±2.96^{e}
T1	403.91±58.2^{a}	162.6±9.33^{a}
T2	205.86±9.27^{b}	100.54±3.64^{c}
T3	154.74±13.85^{c}	86.85±4.08^{d}
T4	185.47±5.54^{bc}	118.6±4.01^{b}
LSD	41.11	8.03

Note: Different letters indicate statistically significant differences p≤0.05

Serum cholesterol and triglycerides

The experimental groups: Serum total cholesterol (TC) and triglyceride (TG) levels in all groups were presented in Table 2, and the analysis of the experimental groups revealed significant differences. The values of TC (403.91±58.2 mg/dL) and TG (162.6 ± 9.33 mg/dL) in the hyperlipidemic control group (T1), for instance, were significantly higher than the lowest values obtained in the negative control group (C1) (TC, 70.94 ± 4.32 mg/dL; TG, 61.52 ± 2.96 mg/dL). This indicates that feeding high-fat diets successfully induced hyperlipidemia. The ethanolic extract of Spirulina platensis (T2) decreased the TC and TG from 433.75 ± 12.73 mg/dL to 205.86 ± 9.27 mg/dL ($p < 0.01$) and from 777.96 ± 48.80 mg/dL to 100.54 ± 3.64 mg/dL ($p < 0.01$), respectively. These

changes were more significant in the nano-formulated Spirulina group (T3), where TC and TG values dropped to 154.74 ± 13.85 mg/dL and 86.85 ± 4.08 mg/dL, respectively. No significant change was observed, and the improvement was moderate in the ZnO-NP treated group (T4): TC was 185.47 ± 5.54 mg/dL, and TG was 118.6 ± 4.01 mg/dL. The data showed that all treatments were significant in response to improvement in lipid profile, but nano-Spirulina extract was the most effective compared to controls. Showing substantial differences between groups, it was obtained an LSD of 41,11 for cholesterol and 8,03 for triglycerides.

Lipid profile

The serum lipid profile is shown in Table 3. During this phase, the mean cholesterol, triglycerides, LDL, and VLDL levels were significantly elevated in the T1 group, while HDL levels were significantly reduced. Cholesterol (403.91 ± 58.2 mg/dL), triglycerides (162.6 ± 9.33 mg/dL), LDL (352.63 ± 56.78 mg/dL), and VLDL (32.52 ± 1.86 mg/dL) were markedly raised, and a marked decrease was recorded in HDL (18.75 ± 2.09 mg/dL). Treatment with Spirulina extracts showed a dose-dependent improvement. Subjects allocated to the nano-formulated extract (T3) revealed the most significant improvement: Cholesterol levels dropped to107.61 ± 16.78 mg/dL, TG to 55.79 ± 5.3 mg/dL, LDL to 172.51 ± 18.53 mg/dL, whereas HDL increased to 33.24 ± 4.17 mg/dL.

Table 3: Effect of different treatments on serum HDL, VLDL, and LDL levels in experimental rat groups (Mean ± SD).

Group	HDL (mg/dL)	VLDL (mg/dL)	LDL (mg/dL)
C	42.5±2.15^{a}	12.30±0.59^{e}	16.14±4.15^{d}
T1	18.75±2.09^{d}	32.52±1.86^{a}	352.63±56.78^{a}
T2	22.91±3.43^{c}	20.10±0.72^{c}	162.8±12.4^{b}
T3	35.41±3.69^{b}	17.37±0.81^{d}	101.96±13.44^{c}
T4	23.33±1.36^{c}	23.72±0.80^{b}	138.42±7.24^{bc}
LSD	4.06	1.607	40.60

Note: Different letters indicate statistically significant differences p≤0.05

Liver function enzymes (ALT and AST)

Table 4 summarizes the effects of the treatments on liver function. The serum AST (105.23 ± 6.73 U/L) and (91.07 ± 7.78 U/L) of the T1 group were significantly elevated compared to the control group (43.07 ± 3.99 and 33.41 ± 2.68 U/L, respectively), suggesting that the liver was damaged as a result of hyperlipidemia. However, in the T3 group (nano-Spirulina), AST (58.57 ± 8.13 U/L) and ALT (53.82 ± 5.89 U/L) activity was significantly lower than the other groups, suggesting hepatoprotective effects. Enzyme levels were reduced across the T2 (ethanolic Spirulina) and T4 (ZnO-NPs) groups, yet T3 caused the greatest difference.

Table 4: Effect of different treatments on serum AST and ALT levels in experimental rat groups (Mean ± SD).

Group	AST (U/L)	ALT (U/L)
C	43.07±4.77^{d}	33.41±6.07^{e}
T1	105.23±6.73^{a}	91.07±7.78^{a}
T2	77.74±5.76^{b}	65.66±0.60^{c}
T3	58.57±8.13^{c}	53.82±5.89^{d}
T4	75.65±7.73^{b}	80.65±4.06^{b}
LSD	10.16	8.23

Note: Different letters indicate statistically significant differences p≤0.05

Oxidative stress markers

Hyperlipidemia is associated with increased oxidative stress. As illustrated by Table 5, the levels of MDA (4.86 ± 0.36nmol/L), which served as a measure for lipid peroxidation, were significantly higher in the T1 group. At the same time, antioxidant markers (GSH (9.15 ± 0.68mmol/L), CAT (0.207 ± 0.03mmol/L), and SOD (1.17 ± 0.11mmol/L)) were significantly lower than in the control group. Treatment with Spirulina, particularly the nano-formulation (T3), restored antioxidant capacity (GSH: 16.26 ± 0.79, CAT: 0.5 ± 0.02, SOD: 2.02 ± 0.09) and decreased MDA (3.16 ± 0.09).

Table 5: Effect of different treatments on oxidative stress markers in experimental rat groups (Mean ± SD).

Group	GSH (mmol/L)	CAT (mmol/L)	SOD (mmol/L)	MDA (nmol/L)
C	22.78±1.23^{a}	0.730±0.03^{a}	3.16±0.011^{a}	2.35±0.05^{e}
T1	9.15±0.15^{d}	0.207±0.01^{e}	1.17±0.06^{e}	4.86±0.36^{a}
T2	13.59±2.07^{c}	0.332±0.03^{c}	1.66±0.07^{c}	3.61±0.28^{c}
T3	16.26±1.93^{b}	0.500±0.01^{b}	2.02±0.05^{b}	3.16±0.09^{d}
T4	15.03±0.94^{bc}	0.290±0.01^{d}	1.40±0.03^{d}	3.99±0.07^{b}
LSD	2.18	0.038	0.116	0.327

Note: Different letters indicate statistically significant differences p≤0.05

Table 6: Effect of different treatments on cardiac injury markers in experimental rat groups (Mean ± SD).

Group	Troponin (U/L)	LDH (U/L)	CK-MB (U/L)
C	34.98±2.17^{a}	245.7±4.79^{e}	123.13±2.77^{e}
T1	10.28±0.71^{d}	511.8±9.89^{a}	204.24±7.46^{a}
T2	16.10±1.36^{c}	396.8±5.01^{c}	160.75±1.13^{c}
T3	24.43±1.93^{b}	299.9±1.24^{d}	139.83±1.07^{d}
T4	14.14±0.72^{c}	428.5±13.24^{b}	174±3.81^{b}
LSD	2.27	12.12	6.04

Note: Different letters indicate statistically significant differences p≤0.05

Cardiac biomarkers

As shown in Table 6, cardiac markers showed a significant increase in serum troponin (10.28 ± 0.71U/L),

LDH (511.8 ± 9.89 U/L), and CK-MB (204.24 ± 7.46U/L) in the hyperlipidemia (T1 group) vs. control group. Nano-Spirulina (T3) treatment afforded the best cardiac protection, as indicated by troponin 24.43 ± 1.93, LDH 299.9 ± 1.24, and CK-MB 139.83 ± 1.07 U/L.

Discussion

FTIR and XRD findings

Results from FTIR indicated structural changes between the nano-formulated Spirulina extract and ethanolic ZnO-only preparations. The displacement and emergence of new bands indicate interactions and the formation of new chemical bonds between Spirulina phytoconstituents and the ZnO nanoparticle surface, possibly influenced by hydroxyl and carboxyl moieties. Successful loading and potential synergistic effects between carrier and bioactive compounds are indicated (Alprol *et al*., 2023). In addition, the FTIR profile of nano-Spirulina showed significantly sharper signals with higher functional group degrees, supporting previous reports indicating the capacity of algae-derived molecules to serve as reducing and stabilizing agents in the production of green nanomaterials (Alprol *et al*., 2023). The absence of some peaks characterized by distinct peaks in the ethanolic extract further corroborates this nanoform's chemical evolution and reactivity. The results further supported this crystalline nature, which confirmed that the developed nano-formulation retained a crystalline structure, as shown by sharp peaks. Gramstad (2014) noted that nano-algal formulations have higher crystallinity and better biological efficiency, which infers good nanoparticle formation and uniformity.

Effect of nano-Spirulina on physiological parameters

Data collection and analysis show that dietary intervention and natural therapeutics strongly impact HFD-induced hyperlipidemia. T1, the hyperlipidemic control group, showed drastic changes in lipid profile, hepatic enzymes, oxidative stress markers, and cardiac markers, emphasizing the adverse consequences of excessive lipid intake.

The most marked elevation of total cholesterol and triglycerides from baseline was observed in the hyperlipidemic group (T1), supported by findings from previous studies, where high-fat diets are known to alter lipid metabolism. Such diets increase intestinal lipid absorption and hepatic lipogenesis but reduce lipid clearance, leading to hypercholesterolemia and hypertriglyceridemia (Sheu *et al*., 2013; Al-Saman *et al*., 2020). In contrast, Spirulina platensis treatment demonstrated strong hypolipidemic activity, as the TC and TG levels were significantly reduced compared with T3 ($p < 0.05$).

The lipid-lowering action of Spirulina may be due to the modulation of lipid metabolism enzymes and inhibition of lipid accumulation by phycocyanin, γ-linolenic acid, and phenolic antioxidants (Ku *et al*., 2015; Deng and Chow, 2010). Increased solubility, availability, and cellular uptake of the nano-formulation might serve as a mechanism for its increased efficacy (Wang *et al*., 2014). Moderate levels improved with ethanolic extract (T2) and ZnO-NPs (T4) in T3. Still, they showed lower levels than T3, indicating how integrating natural bioactives can provide synergistic effects to enhance the activity further with the aid of nano-delivery systems.

The toxic effects observed were antagonized more potently by the administration of Spirulina platensis, especially in nano-formulated form (T3), when compared to both the crude ethanolic extract (T2) and the zinc oxide nanoparticles (T4). The nano-Spirulina group had significantly lower body weight gain, total cholesterol, triglycerides, and LDL and VLDL levels than the control group and higher levels (Table 3). This has been proven with the high levels of phycocyanin, polyunsaturated fatty acids, vitamins (A, C, E), and some essential minerals (zinc, selenium) in Spirulina that have been shown to increase lipid metabolism, reduce appetite core mechanisms, and elicited antioxidant power (Yousefi *et al*., 2018; Jung *et al*., 2019; Hegazi *et al*., 2024). This finding correlates with several previous investigations that found an appetite-suppressing and antilipogenic effect after Spirulina ingestion, primarily owing to the phytochemicals (e.g., phycocyanin and phenylalanine) that boost satiety and suppress fat accumulation (Yousefi *et al*., 2018; Jung *et al*., 2019; Hegazi *et al*., 2024).

The improvement in lipid metabolism also can be a reason for the bioactive substances (like phycocyanin, polyunsaturated fatty acids, and vitamins) in Spirulina regulate fat absorption in the intestines and improve antioxidants, which was already reported (Ku *et al*., 2015; Al-Saman *et al*., 2020; Sheu *et al*., 2013). Given that previously reported, nano-Spirulina markedly improved liver function by decreasing the elevated ALT and AST levels, which generally refers to hepatoprotective. This flavonoid extract is in nanoform, providing enhanced bioavailability and cellular absorption, improving the delivery of the active compound to the hepatic tissues (Cascone *et al*., 2002; Deng and Chow, 2010; Hannan *et al*., 2020).

The liver protective role of Spirulina is associated with its high content of antioxidants such as phycocyanin, vitamin E, and vitamin C, which scavenge free radicals and stabilize cellular membranes (Deng and Chow, 2010; Hannan *et al*., 2020). This confirms oxidative stress status (malondialdehyde (MDA) elevation and decreased antioxidant enzymes activity (GSH, SOD, CAT)) in T1

and its remarkable improvement in T3. The restoration of antioxidant defenses noted is in keeping with the free-radical-scavenging activity of Spirulina pigments, including phycocyanin, β-carotene, and chlorophyll (Kumar *et al*., 2014; Bermejo *et al*., 2008). This confirms Spirulina's strong antioxidant action through enzymatic and non-enzymatic modes (Kumar *et al*., 2014; Bermejo *et al*., 2008).

Nanotechnology also strengthens this protective effect by boosting the delivery and stability of bioactive molecules. Cradle tropnonins (cTnI/cTnT) can also prove valuable in diagnosing myocardial injury at an early stage. In blood, they reflect injury to the myocardium, manifested as damaged heart muscle fibers typically in response to ischemia, oxidative stress, or systemic inflammation (Brush *et al*., 2016).

The values in Table 6 demonstrate a significant reduction in the serum concentrations of troponin I in the hyperlipidemic group (T1) when compared to all other experimental groups. Troponin is a protein component of the contractile machinery of both cardiac and skeletal muscles, and it is normally present in very small quantities in plasma under normal physiological conditions in the absence of cardiovascular damage and normal to moderate skeletal muscle activity (Bogomolova and Katrukha, 2024). However, the decrease in troponin I in T1 might be related to the reduction in skeletal muscle work load and absolute reduction in physical activity observed in these subjects. This lack of activation could be related to the obesity induced by over consumption of lipids (as evidenced by the high lipid profiles and increased body weight in T1 when compared to the other groups). The increase in muscle stiffness in obesity slows the muscle contraction and relaxation rate, which decreases the release of troponin to the extracellular fluid.

Improved renal clearance could present another explanation for the lower troponin I levels. Kidneys are important for removal of the circulating troponin by urinary excretion, and increased troponin filtration has also been observed in hyperlipidemic or obese states (Westreich *et al*., 2022). Also, some studies have reported that troponin may be drawn by lipoprotein molecules present in plasma which could lead to a reduced detectable concentration. Elevated lipid levels, as we have noted in T1, could facilitate such interaction, causing underestimation of plasma concentrations of troponin (Park *et al*., 2014). In addition, hyperlipidemia is associated with several complications, such as changes in the composition of plasma. Elevated levels of plasma lipids can displace plasma volume and change plasma density, and also may influence the precise quantification of analytes like troponin (Kumar and Sathian, 2013).

In contrast, nano-Spirulina (T3) administration significantly reduced heart troponin (24.43 ± 1.93 U/L), indicating myocardial protection. Such a situation is due to certain antioxidant and anti-inflammatory phytochemicals of Spirulina, such as phycocyanin and vitamin E which decrease oxidative myocardial injury (Sheu *et al*., 2013; Wang *et al*., 2014). On the other hand, Spirulina's lipid-lowering effect helps maintain coronary perfusion, thus preventing ischemic damage and subsequent release of troponin (Ama *et al*., 2017; Al-Saman *et al*., 2020).

This suggests that nano-formulation enhances cardiac delivery and the therapeutic potential of PTN (Wang *et al*., 2014). This nano-formulation increased the protective effects as a consequence of bioavailability, cellular targeting, and intracellular delivery of active constituents, translating to a greater reduction of cardiac stress and enzyme leakage rates (Wang *et al*., 2014; Cascone *et al*., 2002). Overall, the findings of this study demonstrate exciting evidence for the bioactivity of nano-formulated Spirulina platensis in diverse therapeutic applications. Regarding dietary-induced hyperlipidemia, it has hypolipidemic, antioxidant, hepatoprotective, and cardioprotective effects. These results further substantiate its potential as a safe and effective natural therapeutic agent, which is augmented by nanotechnology.

Conclusion

These findings confirmed that hyperlipidemia induced by a high-fat diet causes severe metabolic disorders, such as lipid metabolic disorders, liver functioning damage, oxidative stress, and myocardial injury. Nano-formulated Spirulina platensis (T3) provided the most effective therapeutic outcomes of the interventions tested. It led to major decreases in cholesterol, triglycerides, liver enzymes, and cardiac markers, along with increases in HDL and antioxidant defenses. This can be attributed to the synergistic action of the bioactive compounds in Spirulina combined with enhanced bioavailability through nanotechnology. The nanomaterial of Spirulina may prove to be a novel natural approach for the management of hyperlipidemia-associated complications, and this needs to be explored further in a clinical setting.

Declarations

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Ethical approval for this study was granted by the Institutional Review Board (IRB) of the College of Education, University of Al-Qadisiyah, under Reference No. 31, dated January 23, 2025.

Ethical approval

All animal procedures were performed per ethical guidelines and approved by the Institution Animal Care and Use Committee (IACUC) at the University of Al-Qadisiyah.

Declaration of generative AI and AI-assisted technologies in the writing process

No Generative AI and AI-assisted technologies wer used in the writing process.

Conflict of interest

The authors have declared no conflict of interest.

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