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The comparative Effects of Nano and Conventional Spirulina Extracts on Oxidative Stress and Hepatic Biomarkers in Hyperlipidemic male Rats

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Abstract Obesity is a complicated metabolic disorder characterized by abnormal or excessive fat accumulation in the body that may pose a higher risk for chronic diseases. The present study investigates the protective effects of Spirulina platensis extracts (SPEs) from both conventional and nano-formulated extraction against oxidative stress and liver function in rats with hyperlipidemia. Forty male albino rats were divided into five groups. The C group received normal saline only during the observation period. Hyperlipidemic group (T1) was treated with high fat diet (HFD) for 90 days without any medication. Group T2 was treated with the conventional ethanolic Spirulina extract (100 mg/kg/day) during the HFD. Group T3 was also administered Spirulina extract in a nano-form and in the same dose. Group T4 treated with ZnO-NPs (10 mg/kg/day) with HFD. Animals were treated for 30 days after hyperlipidemia induction. The body weight, and serum MDA, liver enzymes (ALT and AST) were significantly increased while antioxidant enzymes (GSH, CAT and SOD) were prominently reduced in T1 group, indicating oxidative damage. Spirulina treatment, particularly in nano-form (T3), resulted in remarkable enhancement in all previously evaluated parameters. The T3 treatment had the highest increment effect followed by the T2 and T4. This study concluded that nano-formulated Spirulina (T3) showed higher protection level compared to conventional form (T2) and ZnO-NPs (T4) implying that nanotechnology increases biological activity of natural antioxidants, especially under conditions associated with lipid accumulation and development of oxidative stress.

Keywords: Spirulina platensis; oxidative stress; liver enzymes; hyperlipidemia; antioxidant biomarkers.

Introduction Hyperlipidemia is defined as an abnormally high concentration of lipids and lipoproteins in the blood. It is a group of heterogeneous disorders that result in the formation of a group of excess lipids in the bloodstream due to an increase in one of the types of lipids, either hypercholesterolemia, hypertriglyceridemia, or both (mixed hyperlipidemia). This increase is associated with the development of atherosclerosis, which is the main cause of coronary heart disease, ischemic heart disease, stroke, and kidney failure, which have witnessed a significant increase in our time (1) .

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 (2, 3). 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 (4). Reports indicate Spirulina has antioxidant, anti-inflammatory, hypolipidemic, immunostimulatory, and hepatoprotective properties (5, 6). 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 Spirulina remain scarce (7). The objective of this study was to investigate the comparative physiological and biochemical impacts of Ethanolic extract of Spirulina platensis, its nanoform and Nano-

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ZnO on hyperlipidemia induced by high fat diet in male rats. The evaluated parameters were as follows: body weight gain, oxidative stress indicators (MDA, GSH, CAT, SOD) and liver functions enzymes (ALT, AST).

Material and Methods

Ethical Approval Statement

This work was accepted by the Medical Ethics Committee, College of Education, Al-Qadisiyah University. All animal studies were performed in compliance with the institutional guidelines and ethical standards. Written and oral informed consent were sought from the responsible academic quarters before the outset of the study.

Experimental Design

The rats were randomly divided, the first group (G1, control group) consisted of eight rats administered normal saline (0.9% NaCl) throughout the duration of the experiment. The second group (G2) included thirty-two rats subjected to a high-fat diet (HFD) supplemented with 30% animal fat for 60 days to induce hyperlipidemia, as described by (8). the animals in G2 were further subdivided into four treatment groups (n=8 per group) as follows:

•T1 (Positive Control Group): Received only the high-fat diet (30% animal fat) for an additional 30 days without any treatment.

•T2: Administered ethanolic extract of Spirulina platensis at a dose of 100 mg/kg body weight via oral gavage daily for 30 days, while continuing on the high-fat diet.

•T3: Received nano-formulated ethanolic extract of Spirulina platensis at the same dose (100 mg/kg) under identical dietary conditions.

•T4: Treated with zinc oxide nanoparticles (ZnO NPs) at a dose of 10 mg/kg body weight via oral gavage daily while maintained on the high-fat diet.

Chemicals and Dose Justification

The Spirulina powder was obtained from a certified local supplier and taxonomically authenticated by specialists in the Department of Biology, University of Al-Qadisiyah. Zinc oxide nanoparticles were acquired and validated using Fourier-transform infrared spectroscopy (FTIR) in the Chemistry Department of the same university.

The dose for the ethanolic extract of Spirulina platensis was selected based on prior studies that demonstrated its physiological efficacy at 100 mg/kg (9, 10). The same dose was applied to the nano-formulated extract. The ZnO NPs dose (10 mg/kg) was determined according to (11). All treatments were administered once daily via oral gavage for a period of 30 days.

Preparation of Conventional and Nano-formulated Ethanolic Extracts

The Spirulina powder was finely ground using an electric mill. The ethanolic extract was prepared following the method outlined by (12), while the nano-formulated extract was synthesized by loading the ethanolic extract onto nanoparticle carriers based on the method reported by (13). The prepared formulations were stored in sterile, sealed plastic containers and used immediately for dosing.

Assessment of Oxidative Stress Markers

Blood samples were collected at the end of the experiment via cardiac puncture under ketamine/xylazine anesthesia. Serum was isolated and used to evaluate oxidative stress biomarkers. Malondialdehyde (MDA) levels were estimated using a modified method described by Guidet and Shah (14). Levels of reduced glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD) were measured using protocols by (150, (16), and (17), respectively.

Liver Enzyme Activity

Serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined using the colorimetric method of Reitman and Frankel (18) to assess hepatic function.

Body Weight Gain Calculation

Body weight gain was assessed by calculating the difference between the final and initial body weights of the animals at the end of the experimental period using the formula:

Weight Gain (g) = Final Body Weight − Initial Body Weight

Statistical Analysis

All data were analyzed using SPSS version 27. One-way analysis of variance (ANOVA) was performed to evaluate differences among groups, followed by the Least Significant Difference (LSD) test to identify statistically significant pairwise comparisons. Results were considered statistically significant at $p \leq 0.05$ (19).

Results and discussion

Body Weight Gain

The results shown in Table (1) reflect the impact of different treatments on the body weight gain of rats exposed to a high-fat diet. The T1 group, which continued to receive the fat-enriched feed without any therapeutic intervention, recorded a significantly high weight gain (208.5 ± 14.05 g), confirming the successful induction of hyperlipidemia. This value was substantially higher than that of the negative control group (C), which recorded a normal physiological weight gain of 97.25 ± 7.55 g.

On the other hand, treatment with either the conventional ethanolic extract (T2) or the nano-formulated extract (T3) of *Spirulina platensis* led to a significant reduction in body weight gain, registering

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52.5 ± 15.63 g and 30.25 ± 3.01 g respectively. These findings suggest that *Spirulina* in both forms has an anti-obesogenic effect, with the nano-form being more effective. Additionally, the group treated with zinc oxide nanoparticles (T4) also showed a significant decline in weight gain (79 ± 22.64 g), although this reduction was less pronounced than that observed in the *Spirulina*-treated groups.

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 (a)	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)	30.75±20.29 (d)	52.5±15.6 3(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: Small Different letters indicate statistically significant differences between treatments p≤0.05
The numbers indicate the mean ± standard error
C: The control group was dosed with physiological saline for the duration of the experiment .
T1: The first treatment represents the group of rats in which Hyperlipidemia induced by feeding on high fat diet.
T2: The second treatment represents the group of rats that were dosed with alcoholic extract of S. Platensis for the duration of the experiment(100ml/kg).
T3: The third treatment represents the group of rats that were dosed with the Nanoalcoholic extract of S. Platensis for the length of the experiment(100ml/kg).
T4: The third treatment represents the group of rats that were dosed with nano-zinc oxide for the duration of the experiment(10 ml/kg).
The statistically significant difference ($p \leq 0.05$) among the groups illustrates that dietary intervention with *Spirulina platensis*, especially in nano-form, effectively suppresses body weight gain in hyperlipidemic rats. The high-fat diet in T1 likely enhanced bile secretion, lipid absorption, and triglyceride deposition in tissues, aligning with prior studies that demonstrated the obesogenic impact of cholesterol- and cholic-acid-enriched diets (20–23).
The anti-obesity effects observed in the T2 and T3 groups can be attributed to *Spirulina's* rich nutritional profile, including amino acids, vitamins, and high-quality proteins superior even to soybean (24, 25). One suggested mechanism involves L-phenylalanine-induced stimulation of cholecystokinin, which suppresses appetite (26). Clinical findings also confirm *Spirulina's* weight-reducing and metabolic benefits in humans (27, 28).The more pronounced effect in the T3 group indicates that nano-formulated *Spirulina* enhances bioavailability and interaction with cellular receptors due to increased surface area, consistent with findings from (29, 30).T4's moderate weight suppression effect may relate to zinc's role in insulin sensitivity and metabolic regulation, although it was less effective than *Spirulina* (31).

Oxidative Stress Biomarkers

Table 2 displays the serum levels of oxidative stress markers. The T1 group exhibited significantly elevated levels of malondialdehyde (MDA: 4.86 ± 0.36 mmol/L), a marker of lipid peroxidation, indicating oxidative damage due to the high-fat diet. Conversely, antioxidant enzyme levels—glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD)—were significantly lower in T1 compared to the control group.
Treatment with *Spirulina* (T2 and T3) and ZnO NPs (T4) reversed these alterations. The nano-form (T3) showed the highest improvements in antioxidant parameters: GSH (16.26 ± 1.93), CAT (0.500 ± 0.01), and SOD (2.02 ± 0.05), while reducing MDA to 3.16 ± 0.09.

Table 2. Oxidative Stress Related Biomarkers (GSH, CAT, SOD, and MDA) in Serum Levels

Group	GSH (mmol/L)	CAT (mmol/L)	SOD (mmol/L)	MDA (mmol/L)
C	22.78±1.2 (3 a)	0.730±0.0 3 (a)	3.16±0.11 (a)	2.35±0.0 5 (e)
T1	9.15±0.15 (d)	0.207±0.0 1 (e)	1.17±0.06 (e)	4.86±0.3 6 (a)
T2	13.59±2.0 7 (c)	0.332±0.0 3 (c)	1.66±0.07 (c)	3.61±0.2 8 (c)
T3	16.26±1.9 3 (b)	0.500±0.0 1 (b)	2.02±0.05 (b)	3.16±0.0 (d) 9
T4	15.03±0.9 4 (bc)	0.290±0.0 1 (d)	1.40±0.03 (d)	3.99±0.0 7 (b)
LSD	2.18	0.038	0.116	0.327

Note: Small Different letters indicate statistically significant differences between treatments p≤0.05

The numbers indicate the mean ± standard error
C: The control group was dosed with physiological saline for the duration of the experiment.
T1: The first treatment represents the group of rats in which Hyperlipidemia induced by feeding on high fat diet.
T2: The second treatment represents the group of rats that were dosed with alcoholic extract of S. Platensis for the duration of the experiment(100ml/kg).

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T3: The third treatment represents the group of rats that were dosed with the Nanoalcoholic extract of S. Platensis for the length of the experiment(100ml/kg).

T4: The third treatment represents the group of rats that were dosed with nano-zinc oxide for the duration of the experiment (10 ml/kg).

The (T1) group's oxidative imbalance may stem from cholesterol-induced ROS generation and subsequent cytokine-mediated neutrophil activation (32). Lipid peroxidation impairs cell membranes, depletes GSH, and reduces enzymatic antioxidant defenses (33). This is consistent with reports from (34, 35). who observed MDA elevation and SOD/CAT suppression in hyperlipidemic rats The restoration of antioxidant levels in Spirulina-treated groups likely results from phycocyanin and carotenoids, which act as potent free radical scavengers and anti-inflammatory agents (36). T3's superior effect reaffirms the therapeutic promise of nano-delivery systems for enhancing cellular uptake (37)

Table (3) illustrates serum transaminase levels. The T1 group showed significantly elevated ALT (91.07 ± 7.78 U/L) and AST (105.23 ± 6.73 U/L), reflecting hepatic injury due to prolonged lipid overload. The control group recorded physiological levels (ALT: 33.41 ± 6.07; AST: 43.07 ± 4.77).

In contrast, T2, T3, and T4 groups demonstrated significant reductions, with the T3 group exhibiting the most pronounced improvement: ALT (53.82 ± 5.89), AST (58.57 ± 8.13). T2 and T4 followed in efficacy.

Table (3). Effect of Different Treatments on Serum AST and ALT Levels in Experimental Rat Groups (Mean ± SD)

Group	ALT (U/L)	AST (U/L)
C	(33 ± 6 .07) e	(43.07± 4.77) d
T1	(91.7±7.78) a	(105.23 ±6.73)a
T2	(65.66 ±0.60) c	(77.74 ± 5.7) b
T3	(53.82±5.89) d	(58.57± 8.13) c
T4	(80.65±4.06) b	(75.65 ±7.73) b
LSD	8.23	10.16

Note: Small Different letters indicate statistically significant differences between treatments p≤0.05
The numbers indicate the mean ± standard error

C: The control group was dosed with physiological saline for the duration of the experiment.

T1: The first treatment represents the group of rats in which Hyperlipidemia induced by feeding on high fat diet.

T2: The second treatment represents the group of rats that were dosed with alcoholic extract of S. Platensis for the duration of the experiment(100ml/kg).

T3: The third treatment represents the group of rats that were dosed with the Nanoalcoholic extract of S. Platensis for the length of the experiment(100ml/kg).

T4: The third treatment represents the group of rats that were dosed with nano-zinc oxide for the duration of the experiment (10 ml/kg).

ALT and AST elevation in T1 reflects hepatocellular membrane damage, enzyme leakage, and hepatic oxidative burden (42–44). Improvement in the *Spirulina*-treated groups likely stem from the stabilization of hepatocyte membranes and reduction of inflammatory mediators, as documented in prior studies (38).

Mazokopakis et al. demonstrated that oral *Spirulina* intake lowered liver enzymes in NAFLD patients (39), and similar effects were observed in animal studies (40, 41). The use of nano-formulation in T3 enhances *Spirulina's* hepatic delivery and therapeutic response, consistent with the benefits of nanoparticle-mediated targeting (42).

Conclusion

The present study proved that the normal and nano-ethanolic extracts of Spirulina platensis ameliorate the body weight, antioxidant capacity and liver functions in hyperlipidemic rats. Regarding both forms, the nano-form exhibited better protection against weight gain, induction of antioxidant enzymes, and regulation of liver markers. These findings highlight the higher efficacy of nanotechnology in enhancing the therapeutic effectiveness of Spirulina in metabolic diseases, such as obesity and hepatic diseases.

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Conflict of interest

No conflict of interest is disclosed by the authors.

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