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Ameliorative effects of *Spirulina* supplementation on high fat and micronutrient deficiency associated complications in wistar rats

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Dietary patterns characterised by excessive fat intake combined with inadequate micronutrient consumption are increasingly associated with metabolic disturbances, including obesity, insulin resistance, dyslipidemia, and organ-level dysfunction. Although *Spirulina* is recognised for its nutrient density and metabolic benefits, its efficacy under the compounded stress of a high-fat diet and concurrent micronutrient deficiency has not been systematically evaluated. Addressing this gap is important for developing nutritional strategies relevant to real-world dietary imbalances. This study investigated the ameliorative effects of *Spirulina* (*Arthrospira*) supplementation in Wistar rats fed either a high-fat (HF) diet or a micronutrient-deficient high-fat diet (HFD). Rats were initially maintained on Control (AIN-93G), HF, or HFD diets for 90 days. Thereafter, HF and HFD groups were subdivided to receive either the same diet or the respective diet enriched with 6.5% *Spirulina* biomass for an additional 60 days. HF and HFD feeding resulted in significant body-weight gain, elevated fasting glucose levels, impaired glucose tolerance, dyslipidemia, and marked histopathological alterations in the liver, heart, kidney, and testis. *Spirulina* supplementation moderated body-weight gain, restored fasting glucose and oral glucose tolerance to control levels, and normalised lipid parameters in both HF- and HFD-fed rats. Histological analyses further confirmed substantial recovery of tissue morphology following *Spirulina* intervention. In summary, *Spirulina* supplementation effectively mitigated metabolic and histological impairments induced by combined high-fat intake and micronutrient deficiency. These findings highlight *Spirulina*'s potential as a functional dietary adjunct for managing complex, diet-induced metabolic disorders.

Keywords: Microalgae, Obesity, Glucose homeostasis, Dyslipidemia, Histology

High-fat diets are commonly used to induce rodent models of metabolic syndrome, insulin resistance, and hyperglycemia[1]. These diets also promote hyperlipidemia[2], ectopic lipid accumulation, and tissue-level histopathological changes[3]. In addition to excessive fat intake, deficiencies in essential micronutrients including zinc, iron and vitamins A, C and E have been linked to altered lipid metabolism, heightened inflammation, and reduced insulin sensitivity[4]. Magnesium deficiency further contributes to β-cell dysfunction, impaired glucose tolerance, and progression toward diabetes[5]. Consistent findings from human and animal studies indicate that diabetic conditions are frequently accompanied by altered micronutrient status, highlighting a strong association between micronutrient deficiency and impaired glucose regulation[4,6–8].

Given these interactions, the combination of a high-fat diet and micronutrient deficiency may exacerbate metabolic dysfunction more severely than either condition alone. Conversely, adequate micronutrient intake has been reported to improve glucose tolerance, modulate immune function, and reduce the risk of diabetes-associated complications[9].

Spirulina (*Arthrospira* sp.) is a nutrient-rich cyanobacterium containing high-quality proteins, γ-linolenic acid, carotenoids, vitamins, minerals[10], phenolic compounds, flavonoids[11], and phycocyanin[12]. Studies have demonstrated its ability to reduce blood glucose levels and improve insulin sensitivity in diabetic models[13], and meta-analyses indicate a significant glucose-modulatory effect in individuals with metabolic syndrome[14].

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The novelty of the present study lies in assessing the ameliorative effects of *Spirulina* under the combined metabolic challenge of a high-fat diet and concurrent micronutrient deficiency, an integrated model that better reflects complex nutritional imbalances. In contrast to earlier studies focusing solely on high-fat or diabetic conditions, the present study demonstrates *Spirulina*'s protective efficacy against dual-induced metabolic and histological alterations. The present study, therefore, investigates *Spirulina*'s potential to mitigate alterations in body weight, glucose metabolism, lipid profile, and tissue histology in Wistar rats fed with a micronutrient-deficient high-fat diet.

Materials and Methods

Spirulina biomass, chemicals and reagents

The spray-dried *Spirulina* biomass powder was obtained from Parry Nutraceuticals, EID Parry (India) Ltd, Chennai, Tamil Nadu, India. The composition analysis of *Spirulina* biomass was carried out at the authors' lab and is presented in Supplementary Table 1. Lard, corn starch, sucrose, and soybean oil were purchased from the local market. Vitamins, choline bitartrate, cellulose, L-cystine, mineral mix, glucose and microtitre plate were procured from Hi-Media (Hi Media, Bangalore, India). Casein was purchased from Casein India Company (Mumbai, India). Tertiary-butylhydroquinone was purchased from SRL Chemicals (Mumbai, India). Biochemical analysis kits were procured from Agappe Diagnostics (Agappe Diagnostics, Kerala, India).

Study design

The ethical clearance for the study was obtained from CSIR-CFTRI, Institutional Animal Ethical Committee (IAEC No: 59), which follows the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals, Registration No: 49/Go/ReBi/s/1999/CPCSEA), Government of India, New Delhi, India. Healthy three-week-old male albino Wistar rats (n=30) weighing around 50 g were obtained from the animal house of the Institute and were housed in polypropylene cages with wire mesh tops and hay bedding under hygienic conditions at room temperature (25 ± 2 °C) with 40 ± 10% humidity and a 12-hour day/night cycle. The rats were initially acclimatised for 1 week, with ad libitum access to water and a normal AIN-93 G diet. After the acclimatisation period, the rats were randomised into three groups and fed for 90 days with the following diets: (1) Control group (n=6) with AIN-93 G diet considered as low fat diet; (2) HF group (n=12) with High fat diet; and (3) HFD group (n=12) with High fat and micronutrient deficient diet (50% reduced micronutrient than control group diet).

Baseline fasting blood glucose levels were measured to confirm diet-induced alterations in glucose metabolism in the HF and HFD groups. Following 90 days of dietary intervention and verification of the metabolic shift, *Spirulina* supplementation was introduced. Subsequently, groups 2 and 3 i.e., HF and HFD groups, were divided equally into two further groups, thereby forming a total of five groups which were fed with the following diets for next 60 days: (1) Group I: Control group (n=6) with standard AIN-93 G diet; (2) Group II: HF group (n=6) with High fat diet; and (3) Group III: HFD group (n=6) with High fat and micronutrient deficient diet (50% reduced micronutrient than control group diet); (4) Group IV: HF+Spi group (n=6) with High fat diet incorporating 6.5% *Spirulina* biomass; and (5) Group V: HFD+Spi group (n=6) with HFD diet incorporating 6.5% *Spirulina* biomass. The rats had *ad libitum* access to water and diet throughout the period of study.

All the diets were prepared in the lab, and the formulation of experimental diets was based on AIN-93 G formulation[15] with modifications in lipid source to provide high fat and a reduction in the micronutrient content by 50% to create micronutrient deficiency. The incorporation of *Spirulina* biomass at 6.5% of the diet was based on a previous study where it was shown that the incorporation of 6.5% of *Spirulina* to the diet would provide the vitamin B_{12} at levels comparable to the control AIN-93 diet[16]. All the diet formulations are presented in Table 1.

The body weight of the rats was carefully monitored and recorded at 30-day intervals throughout the study period using a digital scale. Animals were kept for 8–12 hours fasting before any type of induction/blood collection. At the end of the study (a total of 150 days), rats were euthanised using CO_2. Blood was collected by cardiac puncture and transferred into EDTA tubes and plain tubes for plasma and serum, respectively. Blood samples were centrifuged at 3500 rpm for 5 min at 4 °C to obtain the respective fractions for subsequent analyses.

Oral glucose tolerance test (OGTT)

After four weeks of the treatment period (feeding with *Spirulina* supplemented diet), an oral glucose

Table 1 — Composition of modified AIN-93 G diets

Ingredients g/kg diet	Diet				
	Control	HF	HFD	HF+Spi	HFD+Spi
Cornstarch	397.486	117.486	139.986	84.986	107.486
Sucrose	100	100	100	100	100
Fat (soybean oil)	70	-	-		
Animal fat (lard)	-	350	350	350	350
Dextrinised cornstarch (90–94% tetrasaccharides)	132	132	132	132	132
Casein (> 85% protein)	200	200	200	167.5	167.5
Fibre	50	50	50	50	50
Mineral mix	35	35	17.5	35	17.5
Vitamin mix	10	10	5	10	5
L-cystine	3	3	3	3	3
Choline bitartarate (41.1% choline)	2.5	2.5	2.5	2.5	2.5
tert-Butylhydroquinone	0.014	0.014	0.014	0.014	0.014
Spirulina biomass	-	-	-	65	65

[Control diet: AIN- 93G diet; High Fat (HF) diet: AIN-93 G diet with high (35%) fat; Micronutrient Deficient High Fat (HFD) diet: AIN-93 G diet with high (35%) fat and 50% reduced micronutrients than control diet; *Spirulina* supplemented High Fat (HF+Spi) diet: AIN-93 G diet with high (35%) fat incorporating 6.5% *Spirulina*; *Spirulina* supplemented Micronutrient Deficient High Fat (HFD+Spi) diet: AIN-93 G diet with high (35%) fat and 50% reduced micronutrients than control diet incorporated with 6.5% *Spirulina*]

tolerance test was conducted to evaluate the glucose tolerance of the rats. Rats were fasted overnight for 12 hours and then given a dose of 2 g/kg body weight of glucose via oral gavage. The first blood sample was taken from the tail before the glucose load (at the zero-minute time point) and at 30, 60, and 120 minutes after administration. The blood glucose concentration was measured using a glucometer (GlucoCard, Arkray). With the specific glucose values at each time point, the graph was plotted. The area under the curve (AUC) of OGTT was then calculated using GraphPad Prism version 8.0.2 software.

Biochemical parameters

The plasma glucose levels and serum lipid profile (triglyceride, high-density lipoprotein, HDL and total cholesterol, TC) were analysed using reagent kits (Agappe Diagnostics, Kerala, India). The levels of low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) cholesterol were calculated by using the Friedwald formula[17].

LDL (mg/dL) = Total cholesterol – HDL – (TG/5)
VLDL (mg/dL) = Triglycerides/5

The cardiac risk ratio (CRR)/Castelli's risk Index-I (CR-I) was estimated by the ratio of total cholesterol to HDL, i.e., TC/ HDL.

Triglyceride glucose index (TyG) was calculated using the following formula[18].

$$TyG = \ln\left[fasting\ triglycerides\ \left(\frac{mg}{dL}\right) x\ fasting\ plasma\ glucose\ \left(\frac{mg}{dL}\right)/2\right]$$

Histological analysis

For histological studies, various organs (liver, heart, kidney and testis) were dissected out, washed with 0.9% chilled saline solution and placed in 37% formaldehyde solution. These vital tissues were processed, trimmed, embedded in paraffin, sectioned at a thickness of 4 μm, and stained with hematoxylin and eosin. Stained slides were examined using a light microscope camera (C-7070 Wide Zoom, Olympus).

Statistical analysis

A sample size of six rats per group (n = 6) was selected based on a power analysis using expected differences in body weight (mean difference ~100 g, SD ~50 g), with $\alpha = 0.05$ and power = 0.80. The data were analysed by One-way ANOVA followed by Tukey's multiple comparison test using GraphPad Prism version 8.0.2 software, and a P value < 0.05 was considered statistically significant.

Results

Body weight gain

The rats in all the groups showed a gradual increase in their body weight over time (Fig. 1A). All the experimental groups showed a significantly higher ($P < 0.0001$) final body weight compared to the control group (Fig. 1B). The final body weight showed the maximum weight gain of 471±5 g (final weight 521 ±7g) in high fat (HF) diet group, followed by *Spirulina* supplemented high fat (HF+Spi) diet group with a weight gain of 445±9g (final weight 496±11 g), *Spirulina* supplemented high

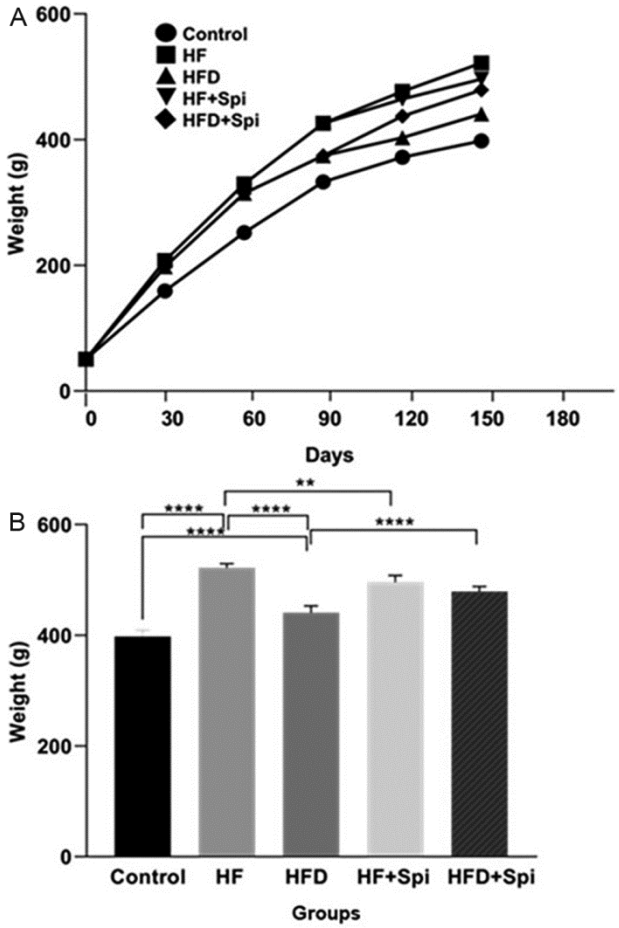

Fig. 1 — Body weight gain of rats (A) Weight gain throughout the study. (B) Final body weight. The results are presented as mean ±SD. Data was analysed using one-way ANOVA (analysis of variance) followed by Tukey's post hoc Multiple Comparisons Test. * P <0.05, ** P <0.01, *** P <0.001, and **** P <0.0001. Control group; HF: high-fat diet group; HFD: micronutrient deficient high-fat diet group; HF+Spi: *Spirulina* supplemented high-fat diet group; and HFD+Spi: *Spirulina* supplemented micronutrient deficient high fat diet group. [Till 90 days, n=12 for HF and HFD groups, thereafter, n=6 for all the groups]

fat deficient diet (HFD+Spi) group with a weight gain of 427±10 g (final weight 478±8g) and high fat deficient diet (HFD) group with a weight gain of 389±10 g (final weight 440 ±11g). The control group showed the lowest weight gain of 347±10g (final weight 397±10 g) among all the groups. Intergroup comparison of final body weight showed the body weight of HF group to be significantly higher (P < 0.0001) than HFD group; HF+Spi group to be significantly lower (P < 0.01) than HF group; and Spi+HFD group to be significantly higher (P < 0.0001) than HFD group. The difference in the final body weight of HF+Spi and HFD+Spi groups was statistically insignificant.

Glycemic profile

The HF and HFD groups showed significantly higher (P < 0.001 and P < 0.0001) fasting blood glucose levels of 118±2 mg/dL and 114±11 mg/dL, respectively, compared to that of the control group (95±4 mg/dL) (Fig. 2A). The fasting blood glucose levels of Spi+HF and Spi+HFD groups were comparable to each other and were also comparable to the control group. In the OGTT, except for HFD group, no other group showed deviation, indicating the synergetic effect of high-fat and micronutrient deficiency in aggravating the imbalance in glucose homeostasis (Fig. 2B). The AUC values of OGTT of HF and HFD groups were significantly higher (P < 0.0001 and P < 0.001) than the control group. The AUC values of OGTT in the HFD+Spi group were significantly lower (P < 0.01) than HFD group and were normalised to the control group. The values in the HF+Spi group were also significantly lower (P < 0.01) than the HF group but remained significantly higher (P < 0.01) than the control group (Fig. 2C).

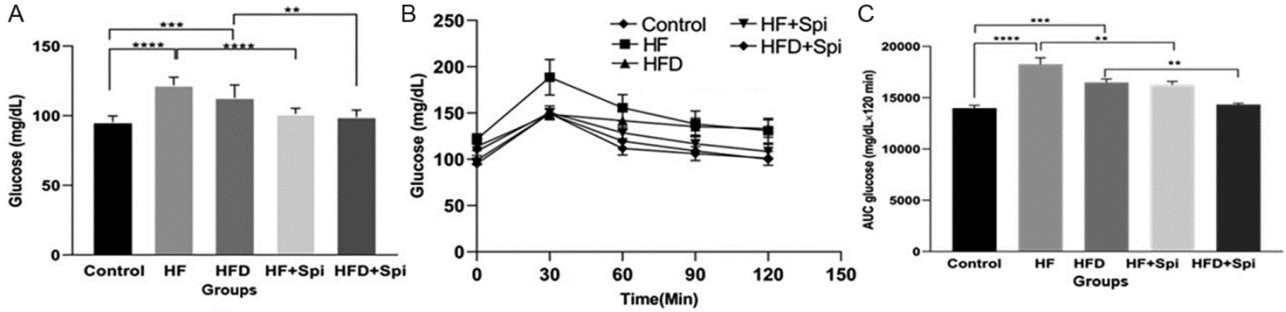

Fig. 2 — Glycemic profile of rats (A) Fasting blood glucose level. (B) Oral glucose tolerance test curve (OGTT). (C) The area under the OGTT curve. The results are expressed as mean ±SD (n=6). Data was analysed using one-way ANOVA (Analysis Of Variance) followed by Tukey's post hoc Multiple Comparisons Test. * P <0.05, ** P <0.01, *** P <0.001, and **** P <0.0001. Control group; HF: high-fat diet group; HFD: micronutrient deficient high-fat diet group; HF+Spi: *Spirulina* supplemented high-fat diet group; and HFD+Spi: *Spirulina* supplemented micronutrient deficient high fat diet group.

Lipid profile

The serum triglyceride values of HF, HF+Spi, HFD and HFD+Spi groups were significantly higher ($P < 0.0001$) than the control group. However, the HF+Spi and HFD+Spi groups showed a significant reduction ($P < 0.0001$ and $P < 0.001$) compared to the value of their corresponding groups without *Spirulina* supplementation, i.e., HF and HFD groups, respectively. The levels of HF+Spi and HFD+Spi were comparable and were normalised to about 40% of the levels of the control group (69 mg/dL) (Fig. 3A). The serum HDL levels of HF and HFD groups were significantly lower ($P < 0.001$) compared to the control group. The serum HDL levels of HF+Spi and HFD+Spi groups were also significantly lower ($P < 0.01$) than the control group, however, they were 1.09-fold and 1.16-fold higher than HF and HFD groups (Fig. 3B). Further, the serum LDL concentrations of HF and HFD groups were significantly higher ($P < 0.01$) than the control group. However, the serum LDL levels of HF+Spi and HFD+Spi groups were comparable to the control group (Fig. 3C). The total serum cholesterol levels in HF and HFD groups were significantly higher ($P < 0.01$) than the control group. However, the levels in HF+Spi and HFD+Spi groups were significantly lower ($P < 0.01$ and $P < 0.05$) than the corresponding HF and HFD groups, respectively and were normalised to the levels of the control group (Fig. 3D). The VLDL levels of HF, HFD, HF+Spi and HFD+Spi groups were significantly higher ($P < 0.001$ and $P < 0.0001$) than the control group. However, the VLDL levels of HF+Spi and HFD+Spi groups were about 30% lower than the HF and HFD groups (Fig. 3E). The cardiac risk ration (CRR) i.e., the ratio of total cholesterol to HDL (TC/ HDL) of HF and HFD groups were significantly elevated ($P < 0.0001$) compared to the control group. However, the HF+Spi and HFD+Spi groups showed significant improvement in the ratio ($P < 0.0001$) compared to HF group and HFD respectively. The triglyceride glucose (TyG) index followed a similar trend as of CRR, with HF and HFD groups showing significant increase compared to the control and *Spirulina* supplemented groups showing significant reduction compared to their corresponding non *Spirulina* supplemented groups (Table 2).

Organ histology

High-fat and micronutrient-deficient diets produced notable pathological changes across organs. Liver tissue analysis of HF and HFD groups showed

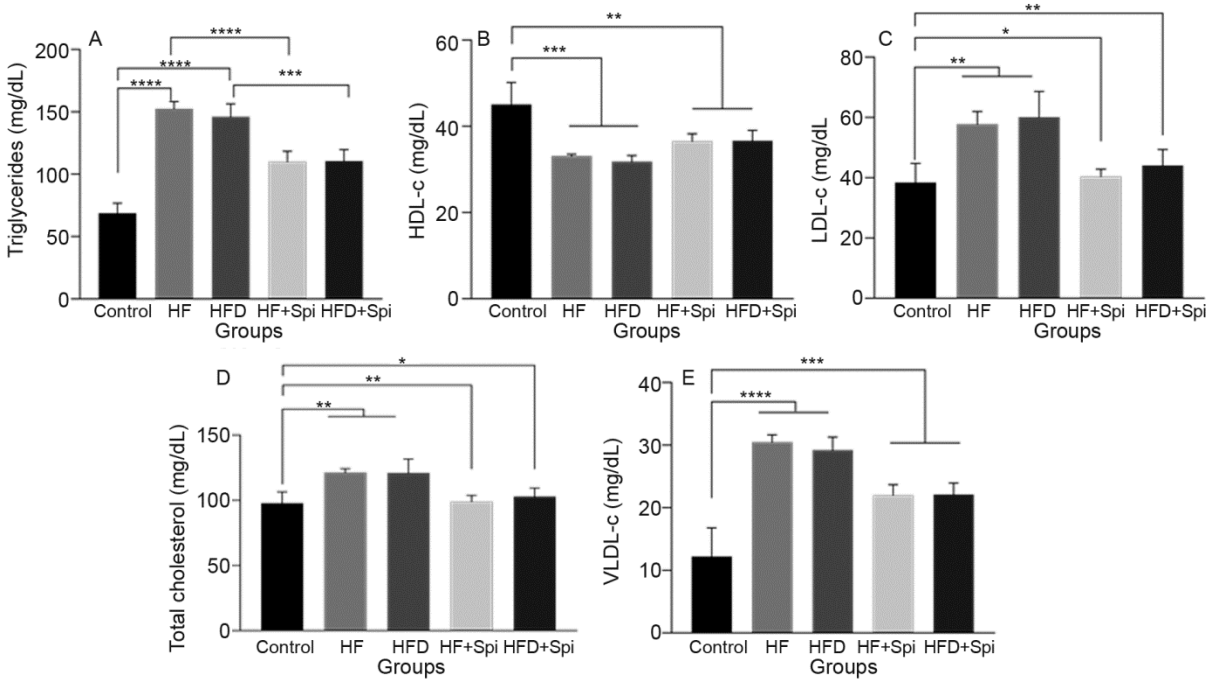

Fig. 3 — Lipid profile of rats. (A) Triglyceride, (B) HDL-c, (C) LDL-c, (D) Total cholesterol, and (E) VLDL-c content. [The results are expressed as mean ±SD (n=6). Data was analysed using one-way ANOVA (Analysis Of Variance) followed by Tukey's post hoc Multiple Comparisons Test. * P <0.05, ** P <0.01, *** P <0.001, and **** P <0.0001. Control group; HF: high-fat diet group; HFD: micronutrient deficient high-fat diet group; HF+Spi: *Spirulina* supplemented high-fat diet group; and HFD+Spi: *Spirulina* supplemented micronutrient deficient high fat diet group]

Table 2 — Cardiac risk ratio (TC/HDL) and triglyceride glucose (TyG) index of rats

Parameters	Groups				
	Control	HF	HFD	HF+Spi	HFD+Spi
Cardiac risk ratio (TC/HDL)	2.09±0.15	4.39±0.04$^{\#\#\#\#}$	3.81±0.30$^{\#\#\#\#}$	2.70±0.06****	2.81±0.21***
TyG index	4.39±0.04	4.89±0.19$^{\#\#\#\#}$	4.85±0.03$^{\#\#\#\#}$	4.65±0.03****	4.65±0.04***

[Values are presented as mean ± SD (n=6). Data was analysed using one-way ANOVA (analysis of variance) with Tukey's Post Hoc Multiple Comparison Test. $^{\#}$Significant difference of HF and HFD groups compared to control; *Significant difference of HF+Spi and HFD+Spi groups compared to their corresponding groups with no *Spirulina* supplementation. # or *P<0.05, ## or **P<0.01, ### or ***P<0.001 and #### or ****P<0.0001]

evidence of hepatic steatosis (a state with too much lipid build-up in the liver). However, the HF+Spi and HFD+Spi groups showed a decrease in hepatic steatosis, and restoration of tissue integrity with the tissue histology comparable to the control group (Fig. 4A). The heart tissues of HF and HFD groups showed focal fatty infiltration in the myocardial cells**.** However, the heart tissues of HF+Spi and HFD+Spi groups were similar to the control group showing reduced pathological changes and normal architecture of the myocardium, with reduced focal fatty infiltration (Fig. 4B). In kidney histology, mild glomerular distension and moderate epithelial cell degeneration were seen in HF and HFD groups, whereas a normal tissue architecture comparable to the control group was observed in HF+Spi and HFD+Spi groups (Fig. 4C). Testis histology showed severe atrophy and degeneration of seminiferous tubules and the destroyed tubular cellular architecture in HF and HFD groups, however the tissue histology was normal and comparable to control group in HF+Spi and HFD+Spi groups (Fig. 4D).

Discussion

High-fat diets disrupt systemic metabolism by increasing caloric excess, promoting lipogenesis, and inducing obesity[19]. The significant weight gain observed in the HF group aligns with well-documented metabolic consequences of high-fat feeding, including adipocyte hypertrophy, mitochondrial dysfunction, and chronic low-grade inflammation[20]. Interestingly A comparatively lower weight gain in micronutrient-deficient high–fat–diet (HFD) fed rats than the HF group, despite identical caloric intake, underscores the essential role of vitamins and trace minerals (B-complex vitamins, vitamin C, vitamin D, as well as minerals such as magnesium and potassium) in energy metabolism[21]. Deficiencies in these nutrients are known to impair ATP generation, reduce anabolic capacity, and increase metabolic stress[22]. The attenuation of body weight loss observed with *Spirulina* supplementation in HFD group in the present study is consistent with earlier findings using *Spirulina* biomass and its various extracts including crude, aqueous, ethanolic, and insulin-like protein extracts[23,24]. *Spirulina* supplementation exerted a dual effect, attenuating excessive weight gain in HF-fed rats and restoring weight gain in HFD-fed rats, reflecting its adaptive metabolic influence. These responses are consistent with previous reports showing that *Spirulina* reduces adiposity by downregulating PPARγ and SREBP-1c signaling[25], enhances fatty acid oxidation through AMPK activation[26].

Spirulina's glucose-lowering effects in both HF and micronutrient-deficient HFD diet groups align with earlier findings[27,28]. Previous studies have shown that *Spirulina* is abundant in proteins, essential fatty acids, vitamins, minerals, and diverse bioactive compounds, all of which contribute to its wide-ranging health benefits and establish it as a highly valued functional food[29,30]. *In silico* docking analysis revealed strong binding affinities of *Spirulina* bioactive compounds with proteins involved in glucose metabolism, supporting their potential role in glycemic regulation[31].

Phycocyanin A phycobiliprotein present in *Spirulina,* inhibits α-amylase, reducing carbohydrate digestion and post-prandial glucose spikes[32]. The PUFA-rich extract of *Spirulina platensis* exhibited anti-diabetic activity by improving glucose tolerance and beneficially altering the composition of the gut microbiota[33]. Also, the sulfated polysaccharide shown to have potent anti-diabetic activity *in vivo* by improving glucose and lipid metabolism, alleviating insulin resistance, and enhancing liver function and antioxidant defenses in STZ-induced diabetic mice[34]. Additionally, *Spirulina* enhances β-cell viability through antioxidant mechanisms[35], including increased glutathione peroxidase and glutathione reductase[36]. Vitamins and minerals such as chromium[37], magnesium[38], folate[39], and Vitamin B_{12}[40]

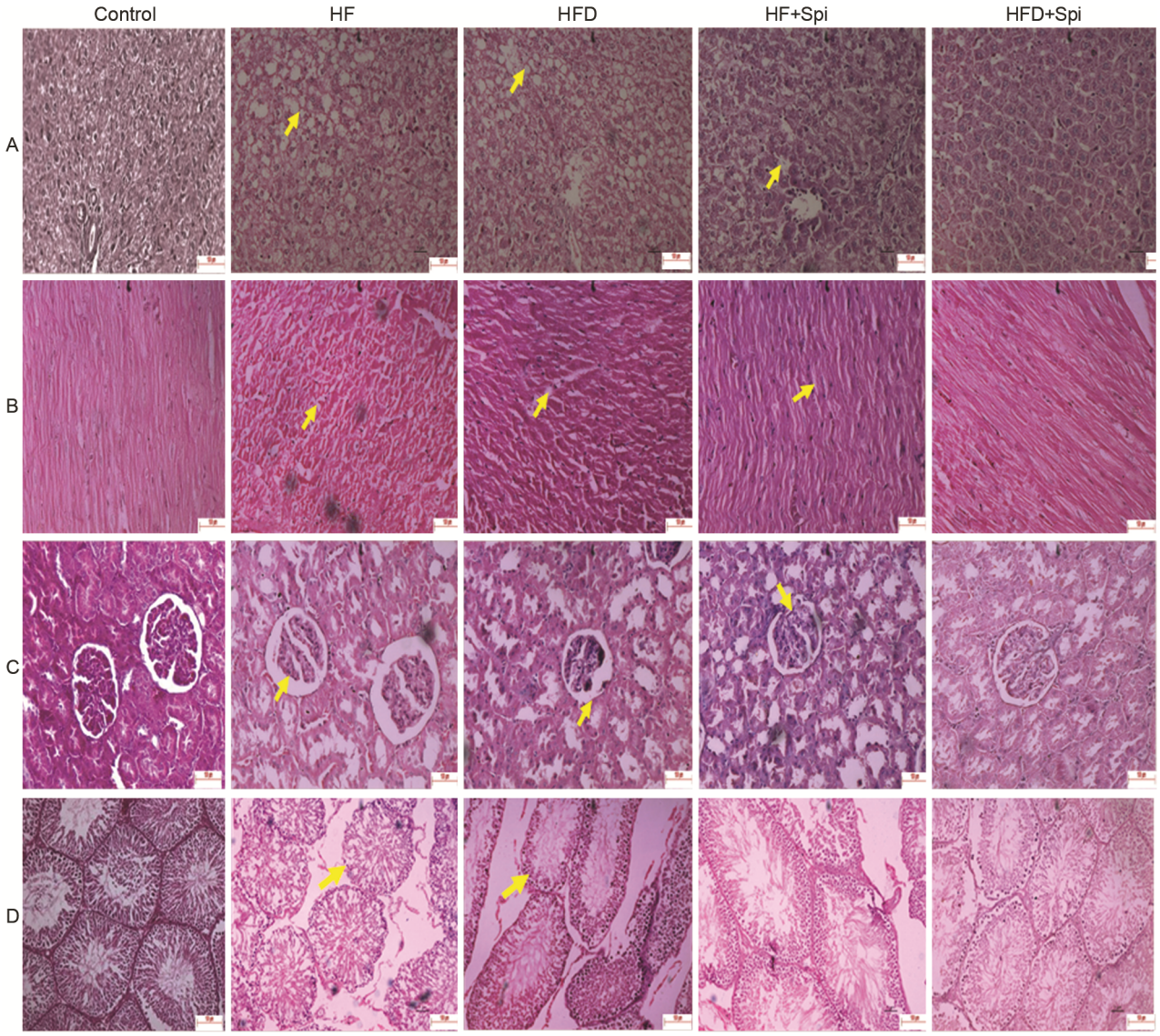

Fig. 4 — Organ histology (20×) of rats. Panels : A: Liver; B: Heart; C: Kidney; D: Testis. Control : Control group showing normal architecture of the liver, heart, kidney and testis (Panels A-D). HF : High-fat diet fed group showing prominent macrovesicular steatosis in the liver (A); focal fatty infiltration in myocardial cells in the heart (B); mild glomerular distention and degeneration of epithelial cells in the kidney (C); and severe atrophy and degeneration of seminiferous tubules in the testis (D). HFD: Micronutrient-deficient high-fat diet fed group showing prominent macrovesicular steatosis in the liver (A); focal fatty infiltration in myocardial cells in the heart (B); mild glomerular distention and degeneration of epithelial cells in the kidney (C); and trophy and degeneration of seminiferous tubules in the testis (D). HF+Spi: *Spirulina* supplemented high-fat diet fed group showing reduced macrovesicular steatosis in the liver (A); reduced focal fatty infiltration in myocardial cells in the heart (B); reduced degeneration of epithelial cells in the kidney (C); and a nearly normal structure of the testis (D). HFD+Spi: *Spirulina* supplemented micronutrient-deficient high-fat diet fed group showing nearly normal architecture of the liver (A); normal heart architecture (B); normal kidney architecture (C); and normal structure of testis (D). The yellow arrow represents specific pathological conditions.

also support glucose metabolism by improving insulin secretion and glucose oxidation. Combined, these mechanisms lead to improved fasting glucose and enhanced glucose tolerance.

The high-fat and micronutrient-deficient high fat diets caused marked dyslipidemia, including elevated triglycerides, LDL, VLDL, and cardiac risk ratio (TC/HDL). *Spirulina* supplementation reversed these disruptions. The hypolipidemic effect is attributed to the inhibition of pancreatic lipase by phycocyanin and the glycolipid H-b2[12], along with the ability of *Spirulina* fibers to enhance bile acid excretion[41]. Another mechanism by which *Spirulina* improves lipid profiles is through the inhibition of HMG-CoA

reductase activity and the activation of the lecithin cholesterol acyltransferase (LCAT) enzyme[42]. Together, these bioactives shift cholesterol transport dynamics toward a more cardioprotective profile.

Histological abnormalities, including hepatic steatosis, myocardial lipid accumulation, renal tubular injury, and testicular degeneration, were pronounced in HF and HFD diet groups, consistent with diet-induced metabolic stress models[43]. *Spirulina* supplementation markedly ameliorated these lesions. *Spirulina*'s hepatoprotective effect may be attributed to methyl donor micronutrients (folate, vitamin B_{12}, choline, methionine), which reduce hepatic lipid accumulation through enhanced VLDL export and homocysteine regulation[44]. A previous report states that *Spirulina* reduces lipid accumulation in the liver by decreasing macrophage infiltration in visceral fat[45]. *Spirulina* also limits oxidative and inflammatory injury in organs through its rich pool of antioxidants[46]. The protective effects on reproductive organs are consistent with previous findings showing *Spirulina* protects against oxidative testicular injury in models of toxin exposure, heavy metal toxicity, and metabolic stress[47–49].

Overall, these findings highlight that *Spirulina* acts through multi-targeted biological pathways, including modulation of insulin signalling, suppression of lipogenesis, enhancement of fatty acid oxidation, stabilisation of antioxidant defences, and preservation of organ histoarchitecture. The integrated action of phycocyanin, PUFA, polysaccharides, vitamins, minerals, peptides, and methyl donors enables *Spirulina* to counteract the combined metabolic burden of high-fat intake and micronutrient deficiency more effectively than single-nutrient interventions. This comprehensive, dual-stressor model strengthens the translational relevance of *Spirulina* as a nutraceutical for complex, diet-induced metabolic disorders.

Conclusion

The present study demonstrates that *Spirulina* effectively moderated body weight gain, normalised fasting glucose levels, and improved glucose tolerance in both HF- and HFD-fed rats. Supplementation also significantly corrected dyslipidemia by reducing triglycerides, LDL, VLDL, and TC/HDL ratios while improving HDL levels. Histological assessments further confirmed that *Spirulina* mitigated hepatic steatosis, reduced myocardial and renal tissue damage, and restored testicular architecture altered by HF and HFD feeding. Overall, the findings clearly demonstrate that *Spirulina* supplementation met the primary objective of the study by counteracting the combined metabolic burden of high-fat intake and micronutrient deficiency. *Spirulina*'s multi-targeted effects on glucose regulation, lipid homeostasis, and tissue integrity highlight its potential as a nutritional intervention for complex diet-induced metabolic disorders.

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Conflict of interest

The authors have no conflict of interest to declare.

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