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Protective Effects of Spirulina Supplementation Against Exercise-Induced Cardiac Remodeling: Integrated Morphological and Biochemical Evidence from an Experimental Study

Jalalova Vazira Zamirovna[1*], Teshaev Shukhrat Jumayevich[2], Iroda Kurbonovna Sa'dulloyeva[3], Mashkhura Muzafarovna Yuldasheva[4], Sharipova Elvina Midatovna[5], Nozima Yuldasheva[6]

[1]Head of the Department of Clinic pharmacology of the Bukhara State Medical Institute named after Abu Ali ibn Sino, PhD, Associate Professor 200126, Gijduvan Str, 23, Bukhara, Uzbekistan. ORCID: https://orcid.org/0000-0001-7792-6766, E-mail: jalalova.vazira@bsmi.uz

[2]Doctor of Medical Sciences, Professor of the Department of Anatomy, Clinical Anatomy and Operative Surgery with Topographic Anatomy at the Bukhara State Medical Institute named after Abu Ali ibn Sino. Bukhara, Uzbekistan. ORCID: https://orcid.org/0009-0002-1996-4275, E-mail: teshayev@bsmi.uz

[3]Head of department (Propaedeutics of children diseases and child neurology) Bukhara State Medical Institute, 200100 Bukhara, Uzbekistan. ORCID: https://orcid.org/0000-0002-7640-4584, E-mail: sadulloyeva.iroda@bsmi.uz

[4]Associate Professor at the Department of Russian Language and Literature, Bukhara State Pedagogical Institute; PhD in Pedagogy; Bukhara, Republic of Uzbekistan. ORCID: https://orcid.org/0000-0002-4048-2775, E-mail: mashhurayuldasheva1973@gmail.com

[5]PhD, Assistant of the Department of Clinical Pharmacology, Bukhara State Medical Institute, Bukhara, Uzbekistan. ORCID: https://orcid.org/0000-0003-2397-1881, E-mail: sharipova.elvina@bsmi.uz

[6]Master's student of the Department of Ophthalmology, Bukhara State Medical Institute named after Abu Ali Ibn Sino, Uzbekistan. ORCID: https://orcid.org/0000-0002-2188-3661, E-mail: klinikfarma@mail.ru

Abstract

Intensive physical exercise induces structural remodeling of the myocardium accompanied by oxidative stress and inflammatory responses, which may impair cardiac adaptation and recovery. This study evaluated the protective effects of Spirulina supplementation on exercise-induced myocardial alterations using morphological and biochemical assessments in an experimental model. Histological examination demonstrated that prolonged intensive exercise caused myocardial fiber disorganization, interstitial inflammatory infiltration, and increased collagen deposition, accompanied by morphometric changes in cardiomyocytes. Digital morphometric analysis revealed a reduction in the proportion of cardiomyocytes (from 38.7% in controls to 28.7% following intensive exercise) and an increase in fibroblast distribution (from 32.3% to 40.3%), indicating early myocardial remodeling. Spirulina

*Corresponding Author: Jalalova Vazira Zamirovna, E-mail: jalalova.vazira@bsmi.uz

supplementation partially restored myocardial architecture, reduced structural damage, improved morphometric parameters, and attenuated biochemical indicators of oxidative stress and tissue injury. These findings suggest that Spirulina exerts cardioprotective effects against exercise-induced myocardial remodeling, most likely through antioxidant, anti-inflammatory, and cytoprotective mechanisms, supporting its potential use as a metabolic support strategy for individuals exposed to intensive physical activity.

Keywords
Spirulina, intensive physical exercise, myocardial remodeling, cardiac morphology, morphometry, oxidative stress, inflammatory response, antioxidant activity, myocardial protection, sports medicine.

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Introduction

Regular physical exercise is widely recognized as one of the most effective non-pharmacological approaches for improving cardiovascular function, metabolic homeostasis, and overall health. Moderate exercise enhances myocardial efficiency, mitochondrial biogenesis, antioxidant defense, and vascular function, thereby reducing the risk of cardiovascular and metabolic diseases. However, when exercise intensity or duration exceeds physiological adaptive capacity, beneficial adaptations may be replaced by maladaptive responses characterized by oxidative stress, inflammatory activation, and structural remodeling of cardiac tissue (Powers et al., 2020; Eijsvogels & Thompson, 2023).

During intensive physical exercise, myocardial oxygen consumption increases several-fold to meet the elevated metabolic demands of contracting cardiac and skeletal muscles. Under these conditions, excessive mitochondrial electron leakage promotes the formation of reactive oxygen species (ROS), including superoxide anions, hydrogen peroxide, and hydroxyl radicals. Although physiological concentrations of ROS participate in intracellular signaling and adaptive responses, sustained overproduction overwhelms endogenous antioxidant defense systems, resulting in oxidative stress, lipid peroxidation, protein oxidation, mitochondrial dysfunction, and DNA damage (Sies, 2020; Xu et al., 2025).

Recent investigations have increasingly demonstrated that oxidative stress represents one of the principal mechanisms responsible for exercise-induced myocardial injury. Excessive ROS generation activates redox-sensitive transcription factors, including nuclear factor-kappa B (NF-κB) and activator protein-1 (AP-1), stimulates pro-inflammatory cytokine production, and induces mitochondrial apoptotic pathways. Consequently, cardiomyocyte degeneration, extracellular matrix remodeling, fibroblast activation, collagen accumulation, and impairment of myocardial contractile function gradually develop under prolonged intensive exercise (Liu et al., 2025; Chandimali et al., 2025).

Inflammation has also emerged as an important contributor to myocardial remodeling associated with excessive physical stress. Experimental and clinical studies indicate that intensive exercise markedly increases circulating concentrations of interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), C-reactive protein, and other inflammatory mediators, which amplify oxidative injury and disrupt normal myocardial repair processes. Persistent inflammatory activation further promotes extracellular matrix deposition, myocardial fibrosis, and deterioration of cardiac microarchitecture (Pedersen, 2019; Fu et al., 2025).

Growing evidence suggests that nutritional interventions rich in natural antioxidants may attenuate oxidative stress while preserving the beneficial physiological adaptations induced by exercise. Functional foods containing biologically active compounds capable of scavenging free radicals, enhancing endogenous antioxidant enzymes, and suppressing inflammatory signaling have therefore attracted considerable interest in sports medicine and cardiovascular research ((Chaouachi et al., 2024; Shiri et al., 2025)).

Among these natural products, Spirulina (Arthrospira platensis) has received increasing scientific attention owing to its exceptionally rich composition of phycocyanin, carotenoids, chlorophyll, phenolic compounds, essential amino acids, polyunsaturated fatty acids, vitamins, and trace elements (Mousavi et al., 2025; Pinto-Leite et al., 2025). These bioactive constituents exhibit potent antioxidant, anti-inflammatory, immunomodulatory, and cytoprotective properties, making Spirulina one of the most extensively investigated nutritional supplements for prevention of oxidative tissue injury. Numerous experimental studies have demonstrated that Spirulina reduces lipid peroxidation, improves mitochondrial function, enhances superoxide dismutase (SOD) and catalase activity, and suppresses

inflammatory cytokine production in various experimental models (Belay, 2023; Lu et al., 2022; Fu et al., 2025).
In recent years, Spirulina supplementation has also been investigated in sports medicine. Clinical and experimental evidence indicates that Spirulina may improve exercise tolerance, accelerate recovery after strenuous physical activity, decrease muscle damage biomarkers such as creatine kinase (CK) and lactate dehydrogenase (LDH), reduce malondialdehyde (MDA) concentrations, and improve antioxidant capacity. These findings suggest that Spirulina may protect highly metabolically active tissues against exercise-induced oxidative injury. Nevertheless, most published investigations have primarily focused on biochemical parameters or exercise performance, whereas considerably less attention has been devoted to the direct evaluation of myocardial structural alterations (Krokidas et al., 2024; Fu et al., 2025).

Related Work

Recent evidence further supports Spirulina as a promising cardioprotective nutraceutical. Recent systematic reviews demonstrated that Spirulina supplementation significantly improves lipid metabolism, reduces systemic inflammation, attenuates oxidative stress, and lowers several cardiovascular risk factors. These beneficial effects appear to be associated with the antioxidant activity of phycocyanin and modulation of inflammatory signaling pathways, thereby supporting its use during conditions characterized by increased oxidative stress, including intensive physical exercise (Shiri et al., 2025; Pinto-Leite et al., 2025; Mousavi et al., 2025).
Over the past decade, increasing attention has been directed toward understanding the molecular and structural mechanisms underlying exercise-induced cardiac adaptation and the potential role of nutritional interventions in preventing excessive myocardial damage. Numerous experimental and clinical studies have demonstrated that while moderate physical activity promotes physiological cardiac remodeling, prolonged or exhaustive exercise may induce oxidative stress, inflammatory activation, mitochondrial dysfunction, and extracellular matrix remodeling, ultimately resulting in structural myocardial alterations (Powers et al., 2020; Eijsvogels & Thompson, 2023).
Several investigators have identified oxidative stress as one of the principal mechanisms responsible for myocardial injury following intensive exercise. Sies (2020) emphasized that excessive production of reactive oxygen species (ROS) disrupts intracellular redox homeostasis and promotes lipid peroxidation, protein oxidation, and DNA damage. More recently, Xu et al. (2025) demonstrated that mitochondrial oxidative stress activates multiple intracellular signaling pathways, including NF-κB, MAPK, and apoptotic cascades, thereby accelerating cardiomyocyte degeneration and inflammatory responses. Similarly, Liu et al. (2025) concluded that persistent oxidative imbalance contributes to myocardial fibrosis and impaired cardiac function through activation of fibroblasts and excessive collagen deposition.
Inflammatory signaling has also been extensively investigated in the context of exercise-induced myocardial remodeling. Pedersen (2019) reported that strenuous exercise markedly increases circulating concentrations of IL-6, TNF-α, and other cytokines that regulate tissue repair and immune adaptation. Although transient inflammatory activation is considered a physiological response to exercise, prolonged elevation of inflammatory mediators may aggravate myocardial injury and delay structural recovery. Recent systematic analyses further confirmed that oxidative stress and inflammation are closely interconnected biological processes that collectively determine the severity of cardiac remodeling (Chandimali et al., 2025).
Natural antioxidant supplementation has emerged as an attractive strategy for reducing exercise-induced oxidative damage without compromising physiological adaptation. Among various bioactive compounds, Spirulina (*Arthrospira platensis*) has attracted considerable scientific interest because of its high concentrations of phycocyanin, carotenoids, chlorophyll, vitamins, essential amino acids, minerals, and polyphenolic compounds. These constituents possess strong antioxidant, anti-inflammatory, and immunomodulatory properties capable of protecting cellular structures against oxidative injury (Belay, 2023).
Numerous experimental studies have demonstrated beneficial effects of Spirulina on oxidative stress biomarkers. Lu et al. (2022), in a systematic review of experimental and clinical investigations, reported that Spirulina supplementation significantly reduced malondialdehyde (MDA) concentrations while increasing endogenous antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). Similar findings were summarized by Fu et al. (2025), whose meta-analysis demonstrated improvements in cardiometabolic health together with attenuation of inflammatory responses following Spirulina supplementation. Furthermore, Krokidas et al. (2024)

observed reduced exercise-induced oxidative stress in physically active individuals receiving Spirulina, indicating its potential role in improving recovery after intensive exercise.

Despite these encouraging findings, the majority of previous studies have primarily evaluated biochemical parameters, antioxidant capacity, exercise performance, and systemic inflammatory markers. Only a limited number of investigations have examined the direct effects of Spirulina on myocardial histological architecture. Most available reports rely on serum biomarkers such as CK, LDH, troponins, or cytokines, whereas detailed histopathological assessment, quantitative morphometry, and immunohistochemical characterization of myocardial tissue remain insufficiently explored.

Another limitation of the current literature is the lack of integrated experimental studies simultaneously evaluating histological changes, morphometric characteristics, biochemical alterations, and apoptotic activity within the myocardium following intensive physical exercise. Although several authors have independently investigated oxidative stress or inflammatory responses, comprehensive correlation between biochemical findings and structural myocardial remodeling has rarely been established. Consequently, the precise tissue-level mechanisms through which Spirulina exerts cardioprotective effects remain incompletely understood.

Moreover, previous investigations have demonstrated considerable heterogeneity regarding exercise protocols, Spirulina dosage, supplementation duration, animal species, and analytical methods, making direct comparison among studies difficult. Differences in exercise intensity, treatment regimens, and outcome measures have contributed to inconsistent conclusions concerning the magnitude of Spirulina-induced cardioprotection.

Therefore, additional experimental studies employing integrated histological, morphometric, immunohistochemical, and biochemical approaches are required to clarify the structural mechanisms responsible for Spirulina-mediated myocardial protection. The present study addresses this knowledge gap by comprehensively evaluating myocardial remodeling induced by intensive physical exercise and determining the protective effects of Spirulina supplementation through combined morphological and biochemical analyses.

Materials and Methods

Study Design. This experimental study was designed to investigate the effects of intensive physical exercise and metabolic stress on myocardial morphology, morphometric characteristics, and biochemical indicators, as well as to evaluate the cardioprotective potential of Spirulina supplementation. The experimental protocol integrated histological, morphometric, immunohistochemical, and biochemical approaches to provide a comprehensive assessment of myocardial structural remodeling and metabolic adaptation. The overall study design was based on the experimental framework presented in the dissertation and adapted to meet the reporting standards of contemporary biomedical journals.

Experimental Animals. The experimental study was performed using healthy outbred white laboratory rats (*Rattus norvegicus*). According to the experimental design, animals representing two age groups (young and mature) were included in the study to evaluate age-dependent differences in myocardial adaptation to physical and metabolic stress. Animals were obtained from a certified breeding facility and maintained under standardized vivarium conditions throughout the experiment. Environmental parameters included a controlled temperature of 20–22°C, relative humidity of 50–60%, and a 12-hour light/12-hour dark cycle. Standard laboratory chow and drinking water were available *ad libitum*.

Before initiation of the experiment, all animals underwent a seven-day acclimatization period to minimize stress-associated physiological variability.
All experimental procedures involving laboratory animals were performed in accordance with internationally accepted principles for the care and use of laboratory animals and complied with institutional ethical requirements described in the dissertation materials.
Experimental Groups. Animals were randomly allocated into experimental groups according to age and treatment conditions. Within each age cohort, three experimental groups were established:
1. Control group – healthy animals receiving standard laboratory diet without intensive exercise or Spirulina supplementation.
2. Exercise-induced stress group – animals subjected to intensive physical exercise designed to induce metabolic stress and myocardial remodeling.
3. Exercise + Spirulina group – animals exposed to the same exercise protocol while simultaneously receiving Spirulina supplementation throughout the experimental period.
This experimental design enabled comparative evaluation of age-related myocardial adaptation, exercise-induced pathological changes, and the potential corrective effects of Spirulina.
Physical Exercise Protocol. Experimental physical loading was induced using a treadmill-based running model developed to simulate chronic high-intensity physical exercise. Exercise intensity and duration were progressively increased throughout the study to produce sustained metabolic overload, increased myocardial oxygen demand, oxidative stress, and adaptive structural remodeling. Continuous monitoring of the animals was performed during each training session to minimize accidental injury and ensure uniform exercise performance among all animals assigned to the exercise groups.
The applied exercise protocol was selected because treadmill running provides reproducible physiological stress and has been extensively used for experimental investigation of cardiovascular adaptation associated with prolonged physical activity.
Spirulina Supplementation. Animals allocated to the treatment groups received Spirulina orally throughout the experimental period. Spirulina administration was performed once daily using a body-weight-adjusted dosage calculated individually for each animal. The selected supplementation regimen was intended to provide systemic antioxidant, anti-inflammatory, and metabolic support without inducing toxic effects. Spirulina was administered during the entire period of intensive physical exercise to evaluate its preventive and protective influence on myocardial remodeling.

Tissue Collection. At completion of the experimental protocol, animals were anesthetized according to institutional ethical recommendations. Following euthanasia, the thoracic cavity was carefully opened, and the heart was excised immediately. Myocardial tissue samples were gently rinsed with physiological saline to remove residual blood before fixation.
Specimens intended for morphological evaluation were immersed in 10% neutral buffered formalin for adequate preservation of tissue architecture. Fixed tissues subsequently underwent dehydration through graded ethanol solutions, xylene clearing, paraffin embedding, and preparation of serial histological sections measuring approximately 4–5 μm in thickness using a rotary microtome.
Histopathological Examination. Following fixation in 10% neutral buffered formalin, myocardial tissue samples were processed according to the standard paraffin histological protocol. Briefly, tissues were dehydrated in ascending concentrations of ethanol, cleared in xylene, infiltrated with molten paraffin, and embedded into paraffin blocks. Serial sections measuring 4–5 μm were prepared using a rotary microtome and mounted on glass slides for histological evaluation.

Routine histological assessment was performed using Hematoxylin and Eosin (H&E) staining. This staining protocol enabled evaluation of the general myocardial architecture, including cardiomyocyte arrangement, sarcoplasmic integrity, nuclear morphology, vascular congestion, inflammatory cell infiltration, interstitial edema, degenerative alterations, and evidence of myocardial regeneration. Histological evaluation was performed independently by experienced morphologists who were blinded to experimental group allocation in order to minimize observer bias.
Special attention was given to the structural organization of myocardial fibers, preservation of cross-striations, nuclear localization, cytoplasmic eosinophilia, interstitial connective tissue expansion, vascular morphology, and inflammatory reactions induced by prolonged physical exercise. Histological observations obtained from control, exercise, and Spirulina-treated animals were comparatively evaluated to determine the protective effects of Spirulina against exercise-induced myocardial remodeling. The histological criteria were selected according to the experimental objectives presented in the dissertation.
Histochemical Evaluation (Van Gieson Staining). To evaluate collagen deposition and connective tissue remodeling, additional histochemical staining was performed using the Van Gieson method. This

staining protocol allowed differentiation between myocardial muscle fibers and collagen bundles, thereby providing quantitative assessment of interstitial fibrosis induced by prolonged physical exercise.

Sections stained with Van Gieson were examined under identical microscopic conditions. Collagen fibers appeared bright red, whereas myocardial muscle fibers demonstrated characteristic yellow coloration, facilitating objective differentiation of fibrotic changes from normal myocardial tissue.
Digital segmentation of collagen-positive areas was subsequently performed using computerized image analysis software. The percentage area occupied by collagen fibers relative to the total tissue area was calculated to quantify exercise-induced extracellular matrix remodeling and the antifibrotic effect of Spirulina supplementation. These analyses were performed in accordance with the morphometric approach described in the experimental study.

Digital Morphometric Analysis. Quantitative morphometric analysis was carried out using QuPath digital pathology software (version 0.4.0). High-resolution digital micrographs were acquired under standardized illumination and magnification conditions before computerized image analysis.
For each specimen, multiple non-overlapping microscopic fields were randomly selected to reduce sampling bias. Tissue segmentation algorithms were applied to identify myocardial tissue boundaries and classify individual cellular components according to morphological characteristics.
The following morphometric parameters were quantitatively evaluated:

- cardiomyocyte transverse diameter (μm);
- myocardial fiber density;
- nucleus-to-cytoplasm ratio;
- capillary density (number/mm²);
- percentage of cardiomyocytes;
- fibroblast population density;
- endothelial cell proportion;
- macrophage infiltration;
- destructive cell population;
- collagen-positive area (%).

Digital polygon segmentation was applied to delineate the analyzed tissue area before automated cellular classification. Quantitative measurements were averaged for each experimental group and subsequently subjected to statistical comparison.
The digital morphometric approach substantially reduced observer-dependent variability and increased reproducibility of myocardial tissue assessment by combining automated image segmentation with manual pathological verification. Similar digital pathology methods were successfully applied throughout the dissertation for evaluation of myocardial structural alterations.
Immunohistochemical Analysis. Immunohistochemical investigation was performed to evaluate apoptosis-related molecular alterations associated with prolonged physical exercise and the potential protective effect of Spirulina supplementation.
Among apoptosis-regulating proteins, Bcl-2 was selected as the principal immunohistochemical marker because of its essential role in maintaining cardiomyocyte survival and inhibiting mitochondrial apoptosis. Expression of Bcl-2 was evaluated in myocardial tissue sections using standard immunohistochemical procedures.
Briefly, paraffin sections were deparaffinized, rehydrated, and subjected to antigen retrieval before incubation with the primary antibody against Bcl-2. Bound antibody complexes were visualized using a horseradish peroxidase-based detection system with 3,3′-diaminobenzidine (DAB) serving as the chromogenic substrate. Sections were counterstained with Mayer's hematoxylin.
Positive immunostaining was assessed semi-quantitatively and verified using digital image analysis. Both staining intensity and the percentage of positively stained cardiomyocytes were recorded. Immunohistochemical findings were interpreted together with histological and morphometric

observations to characterize myocardial adaptive and regenerative responses following intensive physical exercise.
The selection of Bcl-2 as the principal molecular marker was based on the experimental objectives and scientific novelty presented in the dissertation.

Results, Performance Evaluation and Discussion

Baseline Morphological Characteristics of the Myocardium in Control Animals

Histological examination of myocardial tissue obtained from intact control rats demonstrated a well-preserved cardiac architecture without evidence of pathological alterations. Hematoxylin and eosin staining revealed regularly arranged cardiomyocytes forming compact myocardial bundles with characteristic transverse striations and preserved cellular organization (Figure 1). Cardiomyocytes exhibited elongated cylindrical morphology with centrally located oval nuclei containing finely dispersed chromatin and distinct nucleoli. The sarcoplasm demonstrated uniform eosinophilic staining without vacuolar degeneration or structural disruption.
The interstitial connective tissue was thin and uniformly distributed between myocardial fibers without signs of edema, inflammatory infiltration, or excessive extracellular matrix accumulation. Capillary networks were evenly distributed throughout the myocardium and demonstrated normal vascular morphology with intact endothelial lining and patent lumina. No vascular congestion, hemorrhage, or microthrombotic alterations were identified.
Histochemical staining using the Van Gieson method confirmed normal collagen distribution within the myocardial interstitium. Collagen fibers were limited predominantly to the physiological perivascular and endomysial connective tissue compartments without evidence of diffuse or focal myocardial fibrosis (Figure 2). The proportion of collagen-positive area remained within physiological limits, indicating preservation of extracellular matrix homeostasis.
Digital morphometric assessment further confirmed the structural integrity of the myocardium. Quantitative image analysis demonstrated homogeneous cardiomyocyte dimensions, preserved nucleus-to-cytoplasm ratio, physiological capillary density, and normal cellular composition throughout the analyzed myocardial sections (Table 1). Automated tissue segmentation identified cardiomyocytes as the predominant cellular population, whereas fibroblasts, endothelial cells, and resident macrophages were detected in proportions corresponding to normal myocardial histology. No statistically significant differences were observed between animals of the same control cohort.
The baseline myocardial morphology therefore provided a reliable physiological reference for subsequent comparison with animals exposed to intensive physical exercise and Spirulina supplementation.

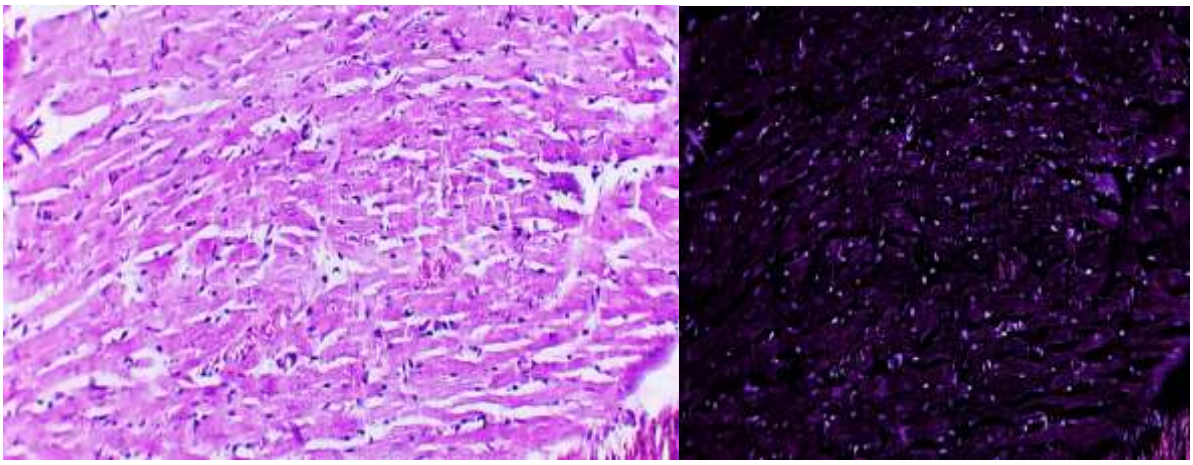

Figure 1. Representative histological and digital morphometric characteristics of the myocardium in 2-month-old control rats.

(A) Representative hematoxylin and eosin (H&E)-stained section demonstrating the normal myocardial architecture characterized by regularly arranged cardiomyocytes with preserved cytoplasmic organization and centrally located nuclei (original magnification ×200).
(B) Digital morphometric analysis of the corresponding myocardial section performed using QuPath software. The analyzed myocardial region is outlined by a red polygon, while the measurement area is highlighted using a blue-black mask. Automated cell classification identified cardiomyocytes as the

predominant cellular population (38.7 ± 1.1%), followed by fibroblasts (32.3%), endothelial cells (8.7 ± 0.4%), destructive cells (3.1 ± 0.7%), and macrophages (1.6 ± 0.8%).
Histological examination of myocardial tissue obtained from 2-month-old control rats demonstrated preservation of normal cardiac architecture without structural abnormalities (Figure 1A). Cardiomyocytes were regularly arranged, exhibiting preserved cross-striations, centrally located nuclei, and homogeneous eosinophilic sarcoplasm. Neither inflammatory infiltration nor interstitial edema or degenerative alterations were observed.
Digital morphometric analysis confirmed the histological observations (Figure 1B). Quantitative tissue segmentation demonstrated that cardiomyocytes constituted the largest cellular population (38.7 ± 1.1%), whereas fibroblasts represented 32.3% of all detected cells. Endothelial cells accounted for 8.7 ± 0.4%, while destructive cells and macrophages comprised only 3.1 ± 0.7% and 1.6 ± 0.8%, respectively, indicating physiological myocardial cellular composition in healthy animals.

Exercise-Induced Histological and Digital Morphometric Alterations in the Myocardium of 2-Month-Old Rats

Histological examination of myocardial tissue obtained from 2-month-old rats exposed to intensive physical exercise and metabolic stress revealed noticeable alterations in myocardial organization compared with the control group (Figure 2A). Although the general arrangement of myocardial fibers remained preserved, focal disturbances in cardiomyocyte alignment and mild disorganization of muscle bundles were observed. Individual cardiomyocytes demonstrated slight variations in cellular morphology, accompanied by moderate widening of the interstitial spaces. No extensive inflammatory infiltration or massive tissue destruction was detected; however, early adaptive structural remodeling of the myocardium was evident following prolonged physical loading.
Digital morphometric analysis confirmed these histological observations (Figure 2B). Automated tissue segmentation demonstrated a reduction in the relative proportion of cardiomyocytes to 28.7 ± 1.1%, compared with the physiological distribution observed in the control myocardium. In parallel, the proportion of fibroblasts increased to 40.3%, indicating activation of the myocardial interstitial compartment. Endothelial cells accounted for 8.5 ± 0.4%, while destructive cells and macrophages represented 3.0 ± 0.9% and 1.7 ± 0.8%, respectively (Table 2). The digital quantitative assessment therefore demonstrated a shift in myocardial cellular composition toward increased stromal cellularity under conditions of prolonged physical and metabolic stress.

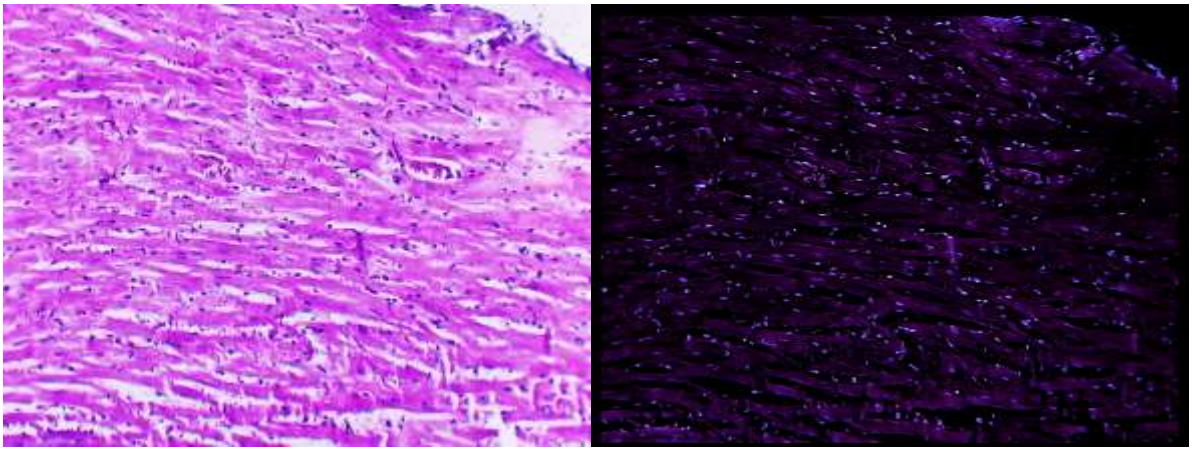

Figure 2. Representative histological and digital morphometric characteristics of the myocardium in 2-month-old rats subjected to intensive physical exercise and metabolic stress.

(A) Representative hematoxylin and eosin (H&E)-stained myocardial section demonstrating mild structural remodeling characterized by focal disorganization of cardiomyocytes and moderate widening of the interstitial spaces (original magnification ×200).
(B) Digital morphometric analysis of the corresponding myocardial section using QuPath software. The analyzed myocardial region is delineated by a red polygon, whereas the measurement area is highlighted using a blue-black mask. Automated cellular classification demonstrated cardiomyocytes (28.7 ± 1.1%), fibroblasts (40.3%), endothelial cells (8.5 ± 0.4%), destructive cells (3.0 ± 0.9%), and macrophages (1.7 ± 0.8%).
Collectively, these findings indicate that intensive physical exercise induced early myocardial structural remodeling characterized by a decreased proportion of cardiomyocytes and a relative increase in

fibroblast density, while the overall myocardial architecture remained largely preserved during this stage of adaptation.

Immunohistochemical Expression of Bcl-2 in the Myocardium of 4-Month-Old Rats Following Spirulina Supplementation

Immunohistochemical evaluation demonstrated detectable Bcl-2 expression in myocardial tissue obtained from 4-month-old rats treated with Spirulina following exposure to intensive physical exercise and metabolic stress (Figure 3A). Positive immunostaining was predominantly localized within the cytoplasm of cardiomyocytes, whereas the remaining myocardial cells exhibited weak or absent immunoreactivity. The distribution of Bcl-2-positive cells was relatively homogeneous throughout the analyzed myocardial sections without evidence of focal staining artifacts.

Digital image analysis performed using QuPath software confirmed the immunohistochemical findings (Figure 3B). A total of 62 cells were identified within the analyzed tissue area, of which 17 cells demonstrated positive Bcl-2 immunoreactivity, whereas 45 cells were classified as negative. Consequently, the overall positive expression rate reached 27.49% within a total analyzed tissue area of 328,644 px² (Table 3). Positive cells were automatically identified and highlighted in red by the digital image analysis algorithm, allowing objective quantitative assessment of Bcl-2 expression.

Compared with myocardial tissue subjected to intensive physical exercise without Spirulina supplementation, Spirulina-treated animals demonstrated a higher proportion of Bcl-2-positive cardiomyocytes together with a corresponding reduction in negatively stained cells. These findings indicate preservation of myocardial cellular viability following Spirulina administration under conditions of prolonged physical and metabolic stress.

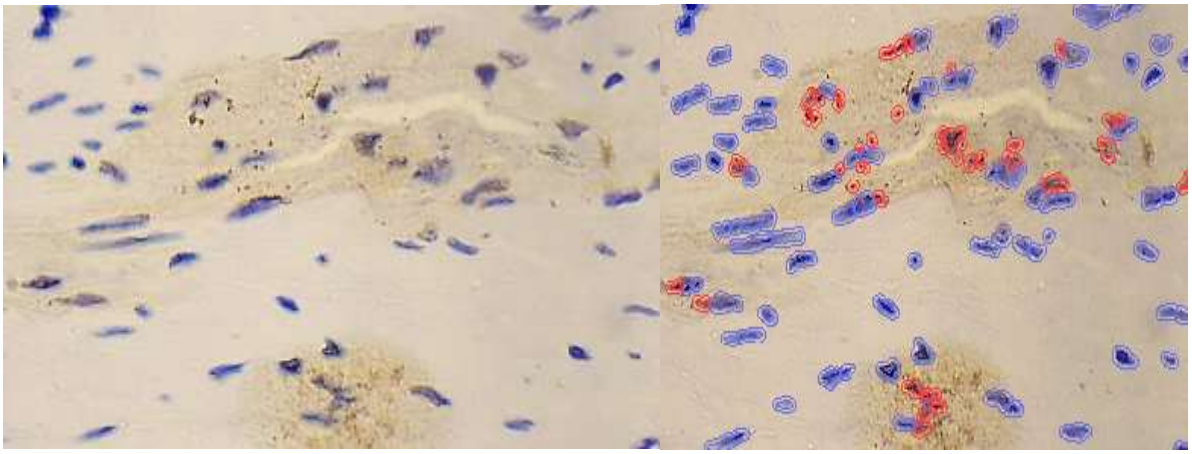

Figure 3. Immunohistochemical expression of Bcl-2 in the myocardium of 4-month-old rats treated with Spirulina after intensive physical exercise and metabolic stress.

(A) Representative immunohistochemical staining for Bcl-2 using the DAB chromogen method (original magnification ×400). Positive cardiomyocytes exhibit brown cytoplasmic staining.

(B) Corresponding digital image analysis performed using QuPath (version 0.4.0). The analyzed tissue area is automatically segmented, and Bcl-2-positive cells are highlighted in red. A total of 62 cells were detected, including 17 positive and 45 negative cells, corresponding to an overall positive expression rate of 27.49%.

The performance of Spirulina supplementation was evaluated using integrated histopathological, digital morphometric, and immunohistochemical parameters obtained from the experimental groups. Histological examination demonstrated that intensive physical exercise and metabolic stress induced structural alterations in myocardial tissue, including changes in cellular organization and an increased proportion of stromal components. Digital morphometric analysis objectively quantified these alterations and confirmed differences in cellular composition among the experimental groups.

Immunohistochemical assessment of the anti-apoptotic marker Bcl-2 further supported the morphometric findings. In the control group, only **3.63%** of the analyzed cells demonstrated positive Bcl-2 immunoreactivity. Exposure to intensive physical exercise and metabolic stress increased the proportion of Bcl-2-positive cells to **32.28%**, indicating activation of cellular adaptive responses under stress conditions. In Spirulina-treated animals, positive Bcl-2 expression remained detectable at **27.49%**, accompanied by preservation of myocardial cellular integrity and reduced numbers of negatively stained cells.

The combined application of digital image analysis and immunohistochemistry provided an objective and reproducible evaluation of myocardial adaptation. QuPath-assisted quantitative analysis minimized observer-dependent variability by automatically detecting positive and negative cells within

standardized tissue regions. This integrated analytical approach strengthened the reliability of morphological assessment and enabled quantitative comparison between the experimental groups.
Overall, the obtained results indicate that Spirulina supplementation was associated with preservation of myocardial cellular viability under conditions of prolonged physical and metabolic stress. The concordance between histopathological observations, digital morphometric measurements, and Bcl-2 immunohistochemical expression demonstrates the usefulness of combining conventional microscopy with artificial intelligence-assisted image analysis for the evaluation of tissue remodeling and cellular adaptation.

Discussion

The present study investigated the structural and cellular adaptations of myocardial tissue following intensive physical exercise and metabolic stress, with particular emphasis on the potential protective role of Spirulina supplementation. By combining conventional histopathology, digital morphometric analysis, and immunohistochemical evaluation of the anti-apoptotic marker Bcl-2, the study provides an integrated assessment of myocardial remodeling under experimental stress conditions.
Histological examination demonstrated that prolonged physical loading induced measurable alterations in myocardial architecture, including changes in cardiomyocyte organization and modifications of the interstitial compartment. These observations are consistent with previous experimental studies reporting that excessive physical exercise promotes myocardial remodeling through increased mechanical load, oxidative stress, and metabolic imbalance. Recent reviews have confirmed that Spirulina supplementation reduces circulating inflammatory cytokines, improves antioxidant capacity, and contributes to cardiovascular protection in both healthy individuals and patients with cardiometabolic disorders, supporting the present experimental findings. Although physiological exercise is generally associated with beneficial cardiac adaptation, chronic or excessive exercise may initiate structural remodeling characterized by cellular stress responses and extracellular matrix reorganization.
Digital morphometric analysis strengthened the conventional histological observations by providing objective quantitative measurements of tissue composition. Compared with control myocardium, the exercise group demonstrated a reduced proportion of cardiomyocytes together with an increased proportion of fibroblasts, suggesting activation of the myocardial stromal compartment during adaptation to prolonged physical stress. The application of QuPath-based digital pathology reduced observer-dependent variability and improved the reproducibility of morphometric assessment, supporting the increasing use of artificial intelligence-assisted image analysis in experimental pathology.
One of the principal findings of this investigation was the alteration of Bcl-2 immunohistochemical expression among the experimental groups. Bcl-2 is recognized as a major anti-apoptotic protein that contributes to mitochondrial membrane stability and promotes cell survival under stressful conditions. The observed differences in Bcl-2 expression indicate that myocardial cells activate endogenous survival pathways in response to prolonged physical and metabolic stress. Similar findings have been reported in experimental models of exercise-induced cardiac remodeling, where modulation of apoptosis-related proteins represents an important adaptive mechanism that limits irreversible cellular injury.
Spirulina supplementation was associated with preservation of myocardial cellular integrity together with detectable Bcl-2 immunoreactivity following prolonged exercise exposure. Although the precise molecular mechanisms were not directly investigated in the present study, these observations are consistent with published evidence demonstrating that Spirulina contains biologically active compounds with antioxidant and cytoprotective properties. Phycocyanin, carotenoids, phenolic compounds, vitamins, and trace elements present in Spirulina have been reported to reduce oxidative stress, stabilize mitochondrial function, and attenuate inflammatory responses in different experimental tissues. The preservation of myocardial morphology observed in the present study is compatible with these previously proposed mechanisms.
The strength of this study lies in the combined application of classical histopathology, quantitative digital morphometry, and immunohistochemistry within a single experimental model. The integration of automated image analysis with conventional microscopic examination provides more objective tissue evaluation than visual assessment alone and may improve the reproducibility of experimental morphological studies.
Several limitations should also be acknowledged. First, the present investigation focused primarily on morphological and immunohistochemical outcomes and did not evaluate molecular signaling pathways involved in apoptosis or oxidative stress. Second, only the Bcl-2 marker was investigated, whereas additional apoptotic regulators such as Bax, Caspase-3, or HIF-1α could provide a more comprehensive

characterization of myocardial adaptation. Finally, longer observation periods and dose-dependent evaluation of Spirulina supplementation would further clarify its cardioprotective potential.
Overall, the present findings support the concept that Spirulina supplementation contributes to preservation of myocardial structural integrity during prolonged physical and metabolic stress. The concordance between histological observations, digital morphometric analysis, and Bcl-2 immunohistochemistry suggests that Spirulina may enhance myocardial cellular resistance to stress-induced remodeling. These findings provide an experimental basis for further investigations into Spirulina as a potential adjunctive strategy for protecting cardiac tissue during conditions associated with increased physiological workload.

Conclusion

The present study demonstrated that prolonged intensive physical exercise and metabolic stress induced structural remodeling of myocardial tissue and altered Bcl-2 immunohistochemical expression. The combined use of histopathology, digital morphometry, and immunohistochemistry provided a reliable and objective assessment of myocardial adaptation.
Spirulina supplementation was associated with preservation of myocardial morphology and maintenance of cellular viability, suggesting a protective effect against stress-induced tissue alterations. In addition, QuPath-assisted digital image analysis improved the objectivity and reproducibility of morphometric evaluation.
Overall, these findings indicate that Spirulina may represent a promising supportive intervention for reducing myocardial damage during prolonged physical and metabolic stress. Further studies involving additional molecular biomarkers and long-term experimental models are required to clarify the underlying protective mechanisms.

Author Contributions
All Authors contributed equally.

Conflict of Interest
The authors declared that no conflict of interest.

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