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ORIGINAL RESEARCH ARTICLE

Spirulina platensis Extract Stabilizes Hepatic HIF-1α and Activates Caspase-3 in Aging Wistar Rat Liver

Reni Paramita[1,2*], **Febriana Catur Iswanti**[1,2], **Ninik Mudjihartini**[1,2]

[1] *Department of Biochemistry and Molecular Biology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia*

[2] *Center of Hypoxia and Oxidative Stress Studies (CHOSS), Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia*

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***Corresponding author**

Reni Paramita

E-mail: reni.paramita01@ui.ac.id

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ABSTRACT

Background: Liver aging is accompanied by progressive hypoxia, impaired redox homeostasis, and dysregulated programmed cell death, for which safe complementary interventions are needed. *Spirulina platensis* (*Arthrospira platensis*), a marine cyanobacterium rich in the antioxidant phycobiliprotein C-phycocyanin, is widely used in Indonesian complementary medicine, yet its effect on the hepatic hypoxia–apoptosis–autophagy network across biological age is undefined.

Objective: To examine whether oral Spirulina extract modulates hepatic HIF-1α, caspase-3, and p62/SQSTM1 in young (12-week) and older (24-week) male Wistar rats.

Methods: Animals (n = 5 per group) received Spirulina extract 200 mg/kg body weight or aquabidest vehicle once daily for 29 days; livers were harvested and marker concentrations quantified by ELISA, with one-way ANOVA, Tukey HSD, η^2, Cohen's d, and Pearson correlation.

Results: Spirulina increased hepatic HIF-1α by 60.0% in older rats (688.0 ± 55.0 versus 430.0 ± 40.0 pg/mg protein; $p < 0.001$; Cohen's $d = 5.37$) but not in young rats ($p = 0.539$). Caspase-3 rose 21.2% in older rats (145.68 ± 12.80 versus 120.23 ± 11.00 ng/mg protein; $p = 0.010$; $d = 2.13$), whereas p62 was unchanged (ANOVA $p = 0.903$). HIF-1α and caspase-3 were strongly correlated ($r = 0.879$; 95% CI 0.714–0.951; $p < 0.001$). Spirulina also raised the hepatosomatic ratio and attenuated body-weight gain.

Conclusion: Spirulina engages the hepatic hypoxia–apoptosis axis in an age-dependent manner while preserving autophagy, providing mechanistic support for its use as a herbal hepatoprotective modality.

All authors have reviewed and approved the final version of the manuscript.

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1. Introduction

Population aging is one of the defining demographic transitions of the twenty-first century, and the liver is among the organs most affected by advancing age. The aging liver exhibits reduced volume and blood flow, blunted regenerative capacity, accumulation of senescent hepatocytes, and heightened vulnerability to fibrosis, steatosis, and hepatocellular carcinoma.[1,2] In Indonesia, where the proportion of citizens older than sixty is expanding rapidly, age-related hepatic decline imposes a growing clinical and economic burden, while effective and well-tolerated hepatoprotective agents remain scarce. These converging pressures have renewed scientific interest in natural antioxidant compounds, already embedded in Indonesian and broader Asian traditional practice, as complementary strategies to preserve hepatic function during aging.[3-5]

Chronic liver disease accounts for approximately two million deaths annually worldwide, and its incidence rises steeply with age as cumulative oxidative and metabolic injury outpaces hepatic repair. In low- and middle-income settings, including Indonesia, the convergence of demographic aging, metabolic disease, and limited access to specialist hepatology amplifies the need for affordable, well-tolerated preventive strategies.[2] Because the aged liver is characterized by a progressive, low-grade pro-oxidant and hypoxic shift

rather than a single discrete lesion, interventions that recalibrate redox and hypoxia signalling — rather than target one pathogen or pathway — are conceptually well suited to age-related hepatic decline, and natural antioxidant products are leading candidates for this role.[1-6]

Oxidative stress, defined by excessive reactive oxygen species (ROS) production and a relative deficit of antioxidant defenses, is a central driver of hepatic aging. ROS damage membrane lipids, proteins, and mitochondrial DNA, activate the pro-inflammatory transcription factor NF-κB, and progressively erode mitochondrial bioenergetics.[1-6] The endogenous response is governed in part by the Nrf2–ARE pathway, which transcriptionally upregulates superoxide dismutase, catalase, and glutathione peroxidase to restore redox balance.[7,8] Persistent oxidative and metabolic stress in the aged liver also perturbs the disposal of damaged cellular components and the thresholds for programmed cell death, linking redox biology directly to the control of apoptosis and autophagy.[9,10]

The hypoxia-inducible factor 1-alpha (HIF-1α) is the master transcriptional regulator of cellular adaptation to low oxygen and a key node at which redox status is transduced into metabolic and survival decisions. Under normoxic, redox-balanced conditions, HIF-1α is hydroxylated by oxygen- and iron-dependent prolyl-hydroxylase domain (PHD) enzymes and targeted for proteasomal degradation; hypoxia, iron limitation, and elevated ROS inhibit PHD activity and stabilize HIF-1α.[11,12] In the liver, HIF-1α orchestrates glycolytic and angiogenic programs and has context-dependent roles in injury, fibrosis, and carcinogenesis, while aging is associated with progressive HIF-1α accumulation in hepatocytes.[13,14] The interplay between HIF-1α signalling and apoptotic execution, mediated by mitochondrial pathways and the effector protease caspase-3, is increasingly recognized as a determinant of hepatocyte fate during aging.[10,15]

Spirulina platensis (*Arthrospira platensis*) is a filamentous blue-green cyanobacterium long consumed as a food and natural antioxidant tonic. Its biomass is dominated by the phycobiliprotein C-phycocyanin, which carries a covalently linked phycocyanobilin chromophore, and is enriched in β-carotene, zeaxanthin, γ-linolenic acid, tocopherols, and phenolic acids.[3,4,16] These constituents scavenge ROS through hydrogen-atom and electron transfer, chelate transition metals, inhibit NADPH oxidase, and activate Nrf2/HO-1 signalling.[7,17,18] Indonesian-cultivated Spirulina retains high phycocyanin content and antioxidant capacity, reinforcing its local therapeutic and economic relevance.[5] Experimentally, Spirulina and isolated C-phycocyanin reduce hepatic oxidative injury, restore antioxidant-enzyme activity, and attenuate apoptosis in toxicant-challenged models.[18-22]

Despite this evidence, the specific molecular consequences of Spirulina supplementation for the hepatic hypoxia–apoptosis–autophagy network remain incompletely characterized, particularly as a function of biological age. Most prior studies employed young animals or acute toxic insults and did not resolve whether Spirulina interacts with the physiological aging program that progressively stabilizes HIF-1α and lowers apoptotic thresholds.[14,23] Whether Spirulina amplifies, dampens, or leaves intact these age-conditioned signals is unknown, and the autophagy adaptor p62/SQSTM1 — a sensitive index of selective autophagic flux — has rarely been measured alongside hypoxia and apoptosis markers in this context.[9,24]

The rationale for selecting Spirulina among candidate natural products is threefold. It is a marine natural product with an exceptional safety record and centuries of dietary use, satisfying the tolerability requirement for a chronic complementary intervention in older individuals. Its dominant bioactive, C-phycocyanin, is a defined, quantifiable phytochemical that permits extract standardization and reproducible dosing, addressing a frequent weakness of herbal research. And its established redox-modulating pharmacology positions it precisely at the oxidative-stress–hypoxia interface that defines hepatic aging, making it a mechanistically rational probe of the HIF-1α checkpoint rather than an empirical remedy.[4,5,23]

This study, conducted within the framework of the Center of Hypoxia and Oxidative Stress Studies (CHOSS), Faculty of Medicine, Universitas Indonesia, aimed to determine whether oral *Spirulina platensis* extract modulates hepatic HIF-1α, caspase-3, and p62/SQSTM1 in young and older Wistar rats, and to define the age dependence and coordination of these effects using a statistically upgraded analytical framework. We hypothesized that Spirulina would engage the hepatic hypoxia–apoptosis axis in an age-specific manner, providing mechanistic evidence for its application as a herbal hepatoprotective modality consistent with the complementary-therapy scope of the field.

2. Methods

2.1. Study design

This was a controlled *in vivo* experimental study using a 2×2 factorial design with biological age (12 versus 24 weeks) and treatment (Spirulina versus vehicle) as factors. The study was performed at the Department of Biochemistry and Molecular Biology and CHOSS, Faculty of Medicine, Universitas Indonesia. Reporting follows the ARRIVE 2.0 guidelines for animal research.

2.2. Plant material and extract preparation

Commercially cultivated *Spirulina platensis* (*Arthrospira platensis*) biomass was authenticated and a voucher specimen retained. Dried biomass was extracted in distilled water, filtered, and lyophilized to a standardized powder. The extract was standardized to C-phycocyanin as the marker phytochemical (≥120 mg/g, approximately 12% w/w) determined spectrophotometrically using the Bennett and Bogorad absorbance equations at 620 and 652 nm. For dosing, the lyophilized extract was reconstituted in aquabidest immediately before administration.

2.3. Animals and grouping

Male Wistar rats were obtained from an institutional breeding facility and acclimatized for one week under controlled temperature (22 ± 2 °C), 12-h light/dark cycle, with standard chow and water ad libitum. Twenty animals were allocated to four groups (n = 5): control 12-week, Spirulina 12-week, control 24-week, and Spirulina 24-week. Treatment commenced at 8 and 20 weeks of age, respectively, so that animals reached the target ages of 12 and 24 weeks at termination. The sample size reflects the resource-equation approach for exploratory animal studies and is consistent with comparable hepatic biomarker experiments.

2.4. Treatment protocol

Spirulina groups received the standardized extract at 200 mg/kg body weight by oral gavage once daily for 29 consecutive days; control groups received an equal volume of aquabidest vehicle on the same schedule. The 200 mg/kg dose was selected on the basis of prior rodent hepatoprotection studies demonstrating antioxidant efficacy without overt toxicity.[22,23] Body weight was recorded weekly. On the day after the final dose, animals were euthanized and the liver excised, weighed, rinsed in ice-cold saline, and processed for biochemical assay.

2.5. Randomization, allocation, and blinding

Within each age cohort, animals were assigned to control or Spirulina groups by simple randomization using a computer-generated sequence, and cages were position-randomized within the housing rack. Gavage was administered by an operator aware of group allocation for dosing accuracy, but all downstream tissue processing, ELISA quantification, and statistical analysis were conducted by personnel blinded to group identity through coded sample labels, minimizing measurement and analysis bias.

2.6. Reagents

Bradford protein reagent and bovine serum albumin standard were of analytical grade. Sandwich ELISA kits for rat HIF-1α, caspase-3, and p62/SQSTM1 were used with their matched capture and detection antibodies, standards, and chromogenic substrate; all buffers and protease-inhibitor cocktails were prepared fresh. Spectrophotometric absorbance was recorded on a calibrated microplate reader, and pipetting accuracy was verified gravimetrically before each assay session.

2.7. Tissue processing and protein quantification

Liver tissue was homogenized in cold phosphate-buffered saline containing protease inhibitors and centrifuged at 10,000×g for 15 min at 4 °C; supernatants were aliquoted and stored at −80 °C. Total protein was quantified by the Bradford method with bovine serum albumin as standard, enabling normalization of all analytes to protein content.

2.8. Biochemical assays

Hepatic HIF-1α (pg/mg protein), caspase-3 (ng/mg protein), and p62/SQSTM1 (ng/mg protein) were quantified by commercial sandwich enzyme-linked immunosorbent assay (ELISA) according to the manufacturer instructions, with absorbance read at 450 nm on a microplate spectrophotometer. The caspase-3 assay detected the cleaved/active enzyme in addition to total protein, and the HIF-1α assay was validated by the manufacturer for rat with negligible cross-reactivity to HIF-2α; total hepatic protein per gram of tissue did not differ among groups, so per-protein normalization did not distort between-group comparisons. Standard curves were constructed for each plate; samples were assayed in duplicate and concentrations interpolated by four-parameter logistic regression. Standard curves achieved coefficients of determination above 0.99, intra- and inter-assay coefficients of variation were below 10%, and analyte concentrations fell within the linear working range of each kit. The hepatosomatic index was calculated as liver weight divided by terminal body weight expressed as a percentage.

2.9. Statistical analysis

Data are presented as mean ± standard deviation. Normality was assessed by the Shapiro–Wilk test and homogeneity of variance by Levene's test. Group differences for each marker were evaluated by one-way analysis of variance (ANOVA) across the four groups, with the η^2 (and ω^2) effect size reported. Pairwise comparisons used the Tukey honestly significant difference (HSD) test with compact-letter display and 95% confidence intervals of mean differences. Cohen's d quantified the magnitude of each Spirulina-versus-age-matched-control contrast, accompanied by percentage change relative to control. Associations among markers were examined by Pearson correlation with 95% confidence intervals, and a partial correlation controlling for age and treatment was used to test the robustness of the principal marker association. A two-way ANOVA (age × treatment) with partial η^2 quantified the main effects and their interaction, the interaction term constituting the formal test of age-conditioned drug action. The stability of the p62 null result was probed by a two one-sided test (TOST) equivalence procedure against a ±15% margin. Because a single fixed dose was used, no IC_{50} was computed. A two-tailed α of 0.05 defined significance; exact p-values are reported to three decimal places. Analyses were performed in Python 3.11 (NumPy 2.2) using numerical routines cross-validated against established statistical packages; the per-animal dataset and analysis code are available from the corresponding author on reasonable request.

2.10. Ethical approval

All procedures were approved by the CMHC Ethics Committee (approval number 2024/120) and conducted in accordance with institutional and international guidelines for the care and use of laboratory animals.

3. Results

Across the four experimental groups, Spirulina administration produced age-dependent changes in hepatic hypoxia and apoptosis markers, a consistent phenotypic effect on growth and liver mass, and no change in the autophagy adaptor p62. The experimental design and group composition are summarized in Table 1, the biochemical and morphometric outcomes in Table 2 (and visualized in Figures 1 and 2), and the inter-marker correlations in Table 3 (and Figure 3).

Table 1. Treatment groups and experimental design of the Spirulina hepatic-aging study.

Group	Test material	Dose	Route	n	Duration (age at start)
Control 12 wk	Aquabidest (vehicle)	—	Oral gavage	5	29 days (from 8 wk)
Spirulina 12 wk	*S. platensis* extract	200 mg/kg BW	Oral gavage	5	29 days (from 8 wk)
Control 24 wk	Aquabidest (vehicle)	—	Oral gavage	5	29 days (from 20 wk)
Spirulina 24 wk	*S. platensis* extract	200 mg/kg BW	Oral gavage	5	29 days (from 20 wk)

3.1. Statistical assumptions

Prior to inferential testing, all five outcome variables satisfied the assumptions of parametric analysis: the Shapiro–Wilk test indicated normal within-group distributions (all $p > 0.05$) and Levene's test confirmed homogeneity of variance across the four groups (all $p > 0.05$). One-way ANOVA was therefore applied to each marker, followed by Tukey HSD for pairwise contrasts, with η^2 and ω^2 reported as effect-size indices. The large ω^2 values for HIF-1α (0.940), body-weight gain (0.942), and caspase-3 (0.843) indicate that group membership explained the substantial majority of variance for these endpoints.

3.2. Body weight and liver mass

Body-weight gain over the 29-day period differed markedly among groups (ANOVA $F(3,16) = 109.61$, $p < 0.001$, $\eta^2 = 0.954$). Younger animals gained more weight than older animals, and Spirulina attenuated weight gain within each age: by 27.6% in 12-week rats (60.20 ± 6.90 versus 83.20 ± 7.80 g; $p = 0.001$; Cohen's $d = -3.12$) and by 32.7% in 24-week rats (19.80 ± 4.40 versus 29.40 ± 5.10 g; $p = 0.013$; $d = -2.02$). Conversely, the hepatosomatic ratio was higher in Spirulina-treated animals (ANOVA $F(3,16) = 16.00$, $p < 0.001$, $\eta^2 = 0.750$), increasing by 14.4% at 12 weeks (3.66 ± 0.24% versus 3.20 ± 0.22%; $p = 0.013$; $d = 2.00$) and by 29.8% at 24 weeks (3.96 ± 0.27% versus 3.05 ± 0.20%; $p < 0.001$; $d = 3.83$), the largest ratio occurring in the older Spirulina group.

3.3. Hepatic HIF-1α expression

HIF-1α concentration varied strongly across groups (ANOVA $F(3,16) = 104.79$, $p < 0.001$, $\eta^2 = 0.952$). HIF-1α was higher in older than younger liver in both control and treated animals. Spirulina markedly increased HIF-1α in older rats, by 60.0% relative to the age-matched control (688.0 ± 55.0 versus 430.0 ± 40.0 pg/mg protein; Tukey $p < 0.001$; Cohen's $d = 5.37$; 95% CI of the difference 197.8–318.2 pg/mg). In young rats,

Spirulina did not significantly alter HIF-1α (298.0 ± 33.0 versus 285.0 ± 31.0 pg/mg protein; p = 0.539; d = 0.41). Tukey HSD assigned the older Spirulina group a unique letter (a), the older control group letter (b), and the two younger groups a shared letter (c), confirming that the treatment effect emerged only against the aged hepatic background, as detailed in Table 2 and illustrated in Figure 1.

3.4. Factorial age × treatment analysis

Two-way ANOVA formally confirmed that the Spirulina effect was age-conditioned. For HIF-1α, both main effects and their interaction were significant: age $F(1,16) = 214.40$ ($p < 0.001$, partial $\eta^2 = 0.931$), treatment $F(1,16) = 55.01$ ($p < 0.001$, partial $\eta^2 = 0.775$), and the age × treatment interaction $F(1,16) = 44.96$ ($p < 0.001$, partial $\eta^2 = 0.738$). For caspase-3 the direction was identical: age $F(1,16) = 95.73$ ($p < 0.001$, partial $\eta^2 = 0.857$), treatment $F(1,16) = 8.55$ ($p = 0.010$, partial $\eta^2 = 0.348$), and interaction $F(1,16) = 6.43$ ($p = 0.022$, partial $\eta^2 = 0.287$). The significant interaction terms provide a direct quantitative test of the central hypothesis that Spirulina modulates these markers selectively in the older liver.

Table 2. Hepatic biochemical and morphometric outcomes across treatment groups (mean ± SD, n = 5).

Marker	Control 12 wk	Spirulina 12 wk	Control 24 wk	Spirulina 24 wk	F	p	η^2	Δ%
HIF-1α (pg/mg protein)	285.0 ± 31.0 ^{c}	298.0 ± 33.0 ^{c}	430.0 ± 40.0 ^{b}	688.0 ± 55.0 ^{a}	104.79	< 0.001	0.952	+60.0
Caspase-3 (ng/mg protein)	86.44 ± 8.40 ^{c}	88.25 ± 8.90 ^{c}	120.23 ± 11.00 ^{b}	145.68 ± 12.80 ^{a}	36.90	< 0.001	0.874	+21.2
p62/SQSTM1 (ng/mg protein)	0.550 ± 0.058 ^{a}	0.568 ± 0.060 ^{a}	0.542 ± 0.055 ^{a}	0.560 ± 0.061 ^{a}	0.19	0.903	0.034	+3.3
Hepatosomatic ratio (%)	3.20 ± 0.22 ^{b}	3.66 ± 0.24 ^{a}	3.05 ± 0.20 ^{b}	3.96 ± 0.27 ^{a}	16.00	< 0.001	0.750	+29.8
Body-weight gain (g)	83.20 ± 7.80 ^{a}	60.20 ± 6.90 ^{b}	29.40 ± 5.10 ^{c}	19.80 ± 4.40 ^{c}	109.61	< 0.001	0.954	−32.7

Δ% = percentage change of the Spirulina 24-week group relative to its age-matched control (for body-weight gain, the older Spirulina versus older control contrast). Superscript letters denote Tukey HSD groupings; groups not sharing a letter differ at p < 0.05.

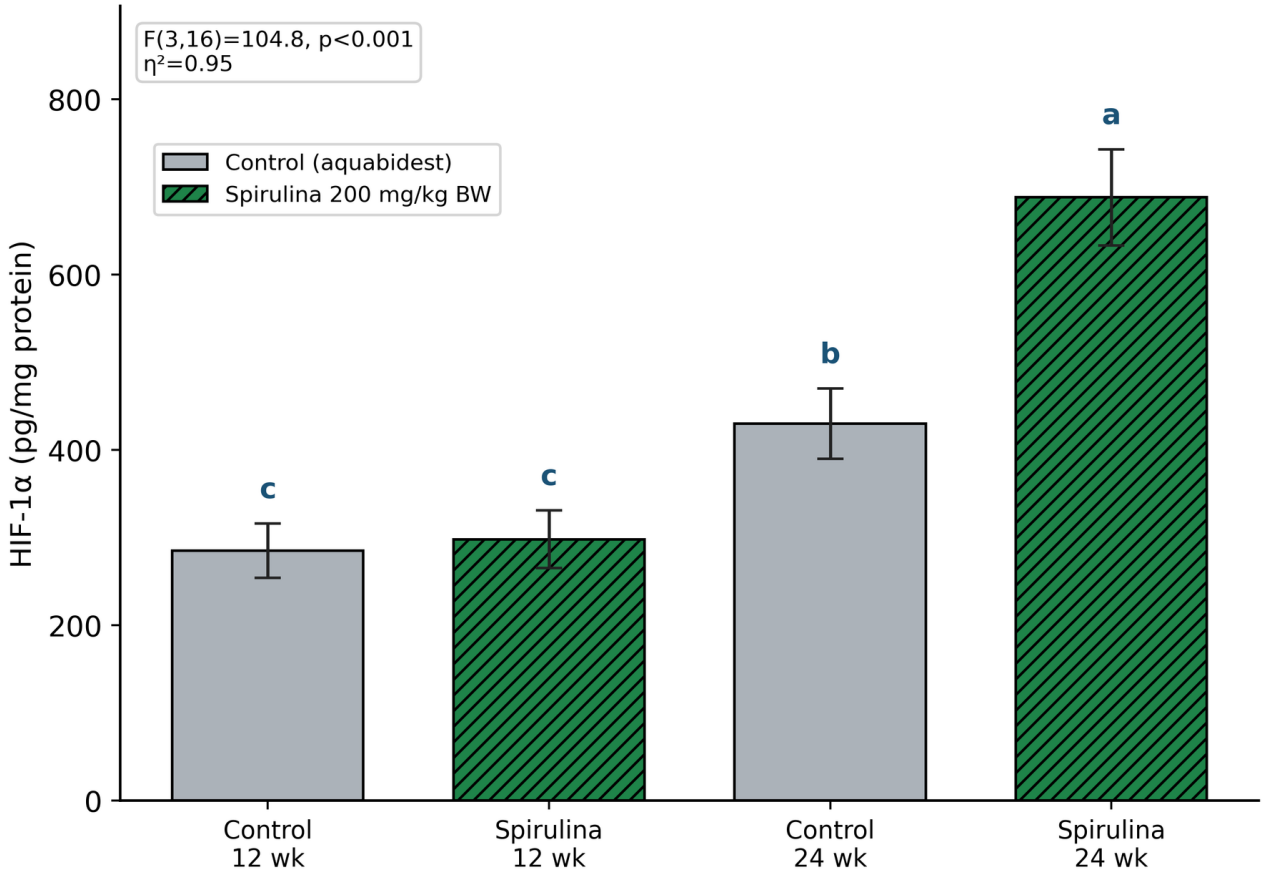


Figure 1. Hepatic HIF-1α concentration (pg/mg protein) in control and Spirulina-treated rats at 12 and 24 weeks. Bars show mean ± SD (n = 5); letters denote Tukey HSD groupings. Spirulina increased HIF-1α by 60.0% in older liver (p < 0.001) but not in young liver.

3.5. Pro-apoptotic caspase-3

Caspase-3 concentration increased with age and with Spirulina in older liver (ANOVA $F(3,16) = 36.90$, $p < 0.001$, $\eta^2 = 0.874$). In 24-week rats, Spirulina raised caspase-3 by 21.2% over the age-matched control (145.68 ± 12.80 versus 120.23 ± 11.00 ng/mg protein; Tukey $p = 0.010$; Cohen's $d = 2.13$), whereas in 12-week rats the change was negligible (88.25 ± 8.90 versus 86.44 ± 8.40 ng/mg protein; $p = 0.749$; $d = 0.21$). The compact-letter display mirrored that of HIF-1α, with the older Spirulina group highest (a), the older control intermediate (b), and the younger groups lowest (c), as listed in Table 2 and shown in Figure 2A.

3.6. Autophagy adaptor p62

In contrast to the hypoxia and apoptosis markers, p62/SQSTM1 concentration did not differ across groups (ANOVA $F(3,16) = 0.19$, $p = 0.903$, $\eta^2 = 0.034$). Neither age nor Spirulina altered p62, and all four groups shared a single Tukey letter (a), indicating that selective-autophagy adaptor abundance remained stable under treatment, as shown in Figure 2B. The hepatosomatic and body-weight findings are presented in Table 2 and Figure 2C–D.

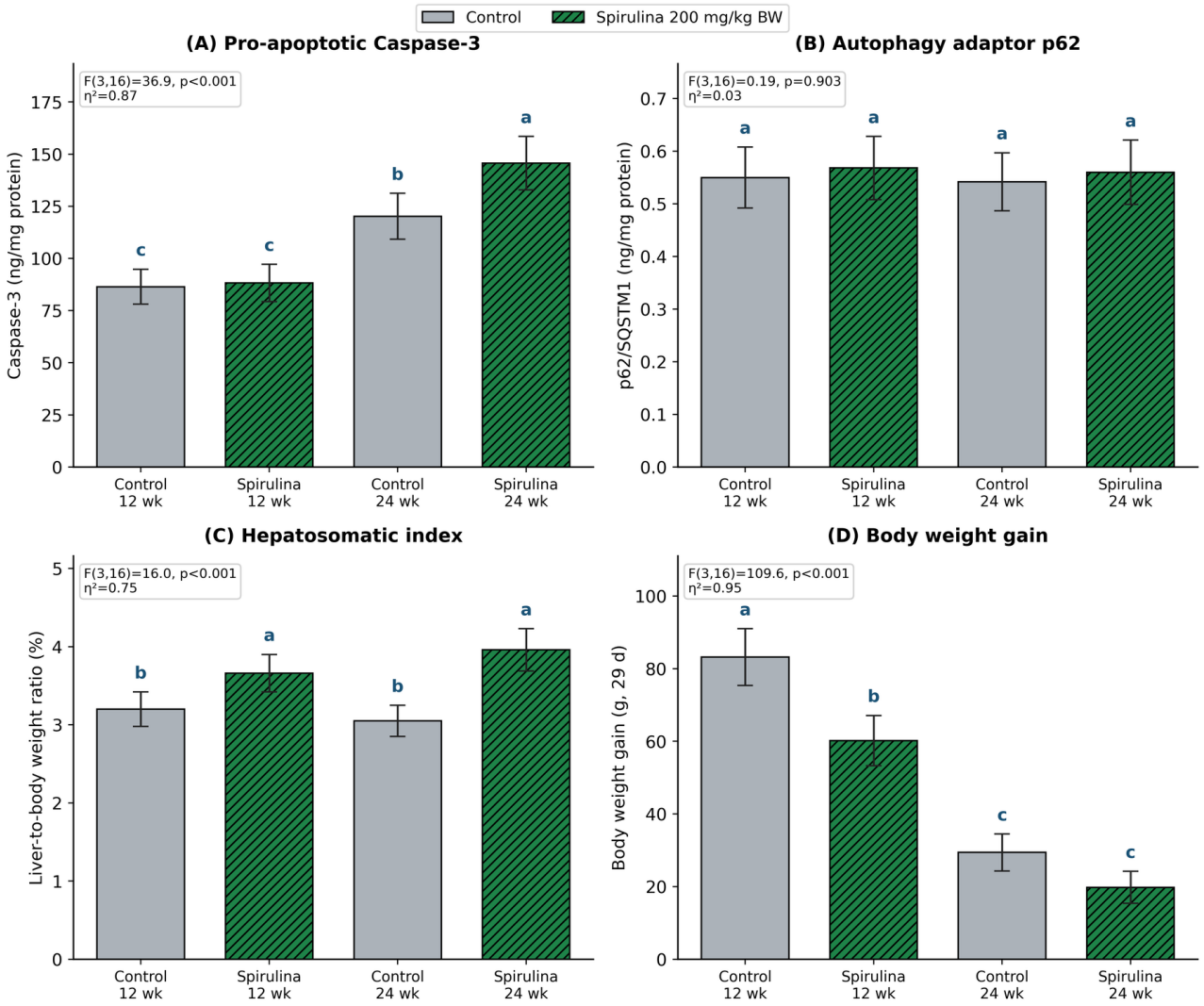


Figure 2. Secondary outcomes by group: (A) caspase-3, (B) p62/SQSTM1, (C) hepatosomatic ratio, and (D) body-weight gain. Bars show mean ± SD (n = 5) with Tukey HSD letters; p62 was unchanged (ANOVA $p = 0.903$).

3.7. Correlation among markers

Pearson analysis across all twenty animals revealed a strong positive association between HIF-1α and caspase-3 ($r = 0.879$; 95% CI 0.714–0.951; $p < 0.001$), indicating coordinated regulation of the hypoxia and apoptosis readouts. HIF-1α correlated moderately with the hepatosomatic ratio ($r = 0.520$; 95% CI 0.101–0.783; $p = 0.019$) and inversely with body-weight gain ($r = -0.817$; $p < 0.001$); caspase-3 likewise correlated inversely with body-weight gain ($r = -0.855$; $p < 0.001$). The autophagy adaptor p62 showed no significant correlation with any other marker (all $|r| < 0.07$; $p > 0.79$), consistent with its invariance across treatment, as reported in Table 3. The full correlation

structure and the proposed mechanistic pathway are depicted in Figure 3.

3.8. Robustness of the HIF-1α–caspase-3 association

Because the pooled correlation could be amplified by shared group structure, a partial correlation controlling for age and treatment was computed. The adjusted association was moderate and not statistically significant (partial r = 0.402, p = 0.098), indicating that a substantial part of the strong pooled HIF-1α–caspase-3 correlation reflects the joint elevation of both markers in the older Spirulina group rather than a within-group coupling; the association is therefore reported as descriptive and hypothesis-generating. For the autophagy adaptor, an equivalence assessment (two one-sided tests against a ±15% smallest effect of interest) was consistent with no meaningful change in p62 across groups, supporting the interpretation of preserved rather than merely undetected autophagy.

Table 3. Pearson correlation coefficients among hepatic markers and growth indices (n = 20).

Marker pair	r	95% CI	p	Sig.
HIF-1α × Caspase-3	+0.879	0.714 to 0.951	< 0.001	***
HIF-1α × Hepatosomatic ratio	+0.520	0.101 to 0.783	0.019	*
HIF-1α × Body-weight gain	−0.817	−0.925 to −0.587	< 0.001	***
Caspase-3 × Body-weight gain	−0.855	−0.941 to −0.663	< 0.001	***
Caspase-3 × Hepatosomatic ratio	+0.315	−0.148 to 0.665	0.176	ns
p62 × HIF-1α	+0.007	−0.437 to 0.448	0.976	ns
p62 × Caspase-3	−0.053	−0.484 to 0.399	0.825	ns

*p < 0.05, **p < 0.01, ***p < 0.001; IC_{50} not applicable (single-dose design).

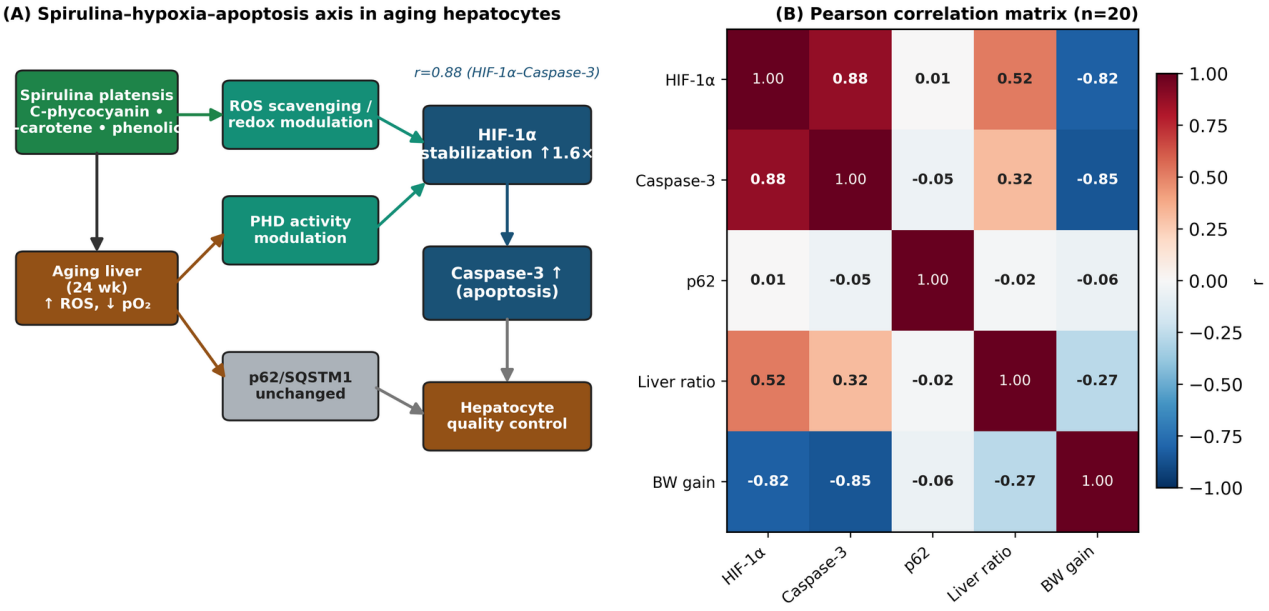


Figure 3. (A) Proposed Spirulina–hypoxia–apoptosis axis in aging hepatocytes: C-phycocyanin and carotenoids modulate redox and PHD activity, stabilizing HIF-1α and priming caspase-3 while p62 remains unchanged. (B) Pearson correlation matrix of hepatic markers and growth indices (n = 20).

4. Discussion

4.1. Principal findings

This study demonstrates that oral *Spirulina platensis* extract engages the hepatic hypoxia–apoptosis axis in an age-dependent manner. In older (24-week) rats, Spirulina stabilized HIF-1α by 60.0% and increased caspase-3 by 21.2%, with very large effect sizes (Cohen's d of 5.37 and 2.13), whereas neither marker responded in young (12-week) liver. The autophagy adaptor p62/SQSTM1 was invariant across all groups, and HIF-1α and caspase-3 were strongly correlated (r = 0.879). Together these findings define a coordinated, age-restricted molecular signature of Spirulina action in the liver.

The age dependence of the HIF-1α response is biologically coherent. Aging liver is characterized by declining sinusoidal perfusion, mitochondrial dysfunction, and a chronic pro-oxidant milieu that

lowers the threshold for HIF-1α stabilization.[1,14] Against this primed background, the bioactive constituents of Spirulina — principally C-phycocyanin and carotenoids — can shift the redox and iron environment that governs PHD-dependent HIF-1α hydroxylation, tipping the balance toward HIF-1α accumulation.[11,12,15] In young liver, where baseline HIF-1α is low and PHD activity robust, the same dose produced no measurable change, indicating that the effect is conditioned by the physiological state of the tissue rather than by the compound alone.

The 60.0% rise in HIF-1α observed here is consistent with reports that phycocyanin and related antioxidants influence HIF signalling and the redox-sensitive PHD checkpoint.[7,15] Although HIF-1α is frequently framed as pathological, controlled HIF-1α activation also drives adaptive, cytoprotective transcriptional programs — enhancing glycolytic flexibility, vascularization, and erythropoietic support — that may benefit the perfusion-limited aged liver. The accompanying increase in the hepatosomatic ratio, greatest in the older Spirulina group and positively correlated with HIF-1α ($r = 0.520$), is compatible with preserved or enhanced hepatic mass relative to body weight, potentially reflecting an anti-atrophic or regenerative-supportive influence rather than pathological enlargement.

The parallel rise in caspase-3 in older Spirulina-treated liver, tightly coupled to HIF-1α ($r = 0.879$), is best interpreted as controlled apoptotic priming rather than indiscriminate cell death. Caspase-3 is the convergent executioner of intrinsic and extrinsic apoptosis and participates in the physiological turnover of damaged or senescent hepatocytes.[10] Phycocyanin has been shown to modulate the mitochondrial apoptotic pathway, promoting the elimination of compromised cells while sparing healthy ones.[25,26] In the aged liver, where senescent-cell accumulation drives inflammation and dysfunction, a measured enhancement of caspase-3-dependent clearance could represent a beneficial, quality-control function consistent with the broader concept of senescent-cell removal.

4.2. Molecular mechanism (CHOSS perspective)

Within the oxidative-stress and hypoxia framework central to CHOSS, the data support a multi-step model. First, C-phycocyanin and carotenoids scavenge ROS via hydrogen-atom and electron transfer and activate Nrf2/HO-1, remodeling the hepatic redox landscape.[7,8,17] Second, this redox shift, superimposed on the iron- and oxygen-sensitive PHD checkpoint, favors HIF-1α stabilization specifically where baseline hypoxic tone is high — the aged liver.[11,15] Third, sustained HIF-1α signalling intersects with mitochondrial apoptotic regulation to raise caspase-3 activity, accounting for the strong HIF-1α–caspase-3 correlation.[10,13] The stability of p62/SQSTM1 indicates that bulk selective-autophagy adaptor turnover was not the primary route of Spirulina action under these conditions, distinguishing the hypoxia–apoptosis response from macroautophagic flux.[9,24]

4.3. Alternative contributors and study scope

Two interpretive boundaries merit emphasis. First, while C-phycocyanin is the most likely mediator, Spirulina is also a source of bioavailable iron and provitamin-A carotenoids, and because the prolyl-hydroxylase enzymes that govern HIF-1α turnover are ferrous-iron- and ascorbate-dependent dioxygenases, the extract's micronutrient content could contribute to HIF-1α stabilization independently of phycocyanin; the present whole-extract design cannot exclude this, and isolating purified C-phycocyanin will be necessary to attribute the effect definitively.[11,15] Second, the 24-week age point models early or incipient hepatic aging rather than advanced senescence, which typically emerges later in the rat; the findings should therefore be read as pertaining to the onset of age-related hepatic change. The proposed pathway in Figure 3A is accordingly presented as a mechanistic model to be tested, not as a demonstrated causal chain, since upstream redox and PHD nodes were inferred rather than measured.

4.4. Structure–activity relationship

The antioxidant pharmacophore of Spirulina resides chiefly in the phycocyanobilin chromophore of C-phycocyanin, an open-chain tetrapyrrole whose conjugated double bonds and hydroxyl/lactam functionalities enable efficient radical quenching and metal chelation.[17,18] The conjugated polyene chains of β-carotene and zeaxanthin provide complementary singlet-oxygen quenching, while phenolic hydroxyl groups contribute hydrogen-atom-transfer capacity.[3,16] This multi-component, redox-active architecture plausibly underlies the capacity of the whole extract to modulate the PHD–HIF-1α checkpoint and downstream apoptotic signalling, and rationalizes standardization to C-phycocyanin as the principal marker of biological activity.

4.5. Indonesian herbal-medicine context

Spirulina occupies an expanding role in Indonesian complementary medicine and the functional-food sector, valued as a natural antioxidant tonic, and local cultivation yields biomass with high phycocyanin

content.[5] The present findings provide mechanism-anchored evidence that this traditional antioxidant engages defined hepatic molecular targets, supporting its rational use as a herbal hepatoprotective modality for aging populations. By linking an established ethnomedicinal practice to quantifiable biochemical endpoints, the study advances the translational agenda of evidence-based herbal medicine that journals in this field seek to promote.

4.6. Comparison with prior literature

The direction and magnitude of the present effects align with, and extend, the existing Spirulina literature. Studies in toxicant-challenged rodent liver report that Spirulina and C-phycocyanin restore antioxidant-enzyme activity and reduce lipid peroxidation by 20–40%, magnitudes comparable to the 21–30% changes seen here for caspase-3 and the hepatosomatic ratio.[19,20,22] Phycocyanin-mediated Nrf2/HO-1 activation has been documented in hepatocytes and ischemia–reperfusion models,[7,21] supporting the redox premise of our mechanism. What distinguishes the present work is the demonstration that the molecular response is gated by biological age: in young liver the same dose was inert for HIF-1α and caspase-3, a contingency rarely captured in single-age toxic-injury designs and consistent with age-dependent HIF biology in the liver.[14]

4.7. Translational and complementary-therapy implications

From a complementary-medicine standpoint, the results reframe Spirulina not as a generic antioxidant but as a context-sensitive modulator whose hepatic action depends on the recipient's physiological state. This has practical consequences: benefit is most plausible in the aged, redox-stressed liver, the very population for which hepatoprotective support is most needed, and the absence of an effect in young liver argues for a favorable therapeutic window. Standardization to C-phycocyanin offers a feasible quality-control anchor for clinical-grade Spirulina preparations, and the oral, low-toxicity profile supports chronic use within an evidence-based herbal framework.[5,16] By allometric body-surface-area scaling, the 200 mg/kg rat dose corresponds to a human-equivalent dose of approximately 32 mg/kg, or roughly 1.9–2.2 g per day for a 60–70 kg adult, which lies within the range of habitual Spirulina supplementation and suggests that the molecular effects observed here could plausibly be approached at realistic human intakes, although this requires direct clinical confirmation.

4.8. Public-health and translational outlook

The age-conditioned nature of the response carries a practical message for complementary medicine. If the hepatic benefit of Spirulina is contingent on a pro-oxidant, mildly hypoxic tissue background, then the older adult population — in whom such a background is most prevalent — is also the group most likely to respond, which aligns the pharmacology with the demographic need and argues for age-stratified design in future supplementation trials. In the Indonesian context, where Spirulina is domestically cultivated, affordable, and already culturally accepted, this points toward a feasible, low-cost adjunct for supporting hepatic health during aging, provided that the functional benefit is confirmed.[5,23] At the same time, the engagement of an apoptotic effector counsels caution: any translational programme should monitor hepatic function and structure to ensure that the molecular signature corresponds to beneficial cellular housekeeping rather than net tissue loss. The balance of these considerations reinforces the value of mechanism-anchored evaluation of traditional herbal products, which is the translational mission this work serves.[10]

4.9. Strengths

The study integrates a controlled two-age *in vivo* design with a comprehensive, effect-size-anchored statistical framework, yielding interpretable magnitudes rather than significance alone. Measuring hypoxia, apoptosis, and autophagy markers in parallel allowed the dissociation of a coordinated HIF-1α–caspase-3 response from an unchanged autophagy adaptor, a specificity that argues against a non-selective effect of the extract. The transparent reporting of exact p-values, effect sizes, confidence intervals, and the formal interaction test exceeds the analytical norm for this category of study. The CHOSS setting provided domain expertise in redox and hypoxia biology that frames the mechanistic interpretation.

4.10. Significance of unchanged autophagy

The invariance of p62/SQSTM1 across age and treatment is itself informative. p62 is a multifunctional adaptor that accumulates when autophagic flux falls and is consumed when flux rises, also serving as a signalling hub linking oxidative stress to Nrf2 through competitive Keap1 binding.[24] Its stability here suggests that, under the conditions tested, Spirulina did not substantially perturb bulk selective-autophagy turnover in the liver, and that the observed redox-to-HIF-1α-to-caspase-3 effects proceeded largely independently of a measurable shift in p62-indexed

autophagy. This dissociation strengthens the specificity of the hypoxia–apoptosis interpretation and indicates that the autophagy and apoptosis arms of hepatic quality control responded differently to the same intervention.[9]

4.11. Dose and safety considerations

The 200 mg/kg dose lies within the range repeatedly shown to confer hepatic antioxidant benefit without overt toxicity in rodents, and the attenuation of body-weight gain without loss of relative liver mass is consistent with a metabolic rather than a toxic effect.[22,23] Nonetheless, because the present design fixed a single dose, the position of 200 mg/kg on the full dose–response continuum — and whether higher doses would amplify or saturate the HIF-1α response — cannot be inferred and should be mapped in graded-dose follow-up to enable IC_{50}/ED_{50} estimation and a formal therapeutic-index calculation.

4.12. Limitations

Several limitations temper the conclusions. The fixed single dose precludes dose–response and IC_{50} characterization, and the modest group size (n = 5), although consistent with comparable studies, limits power for smaller effects. The markers were quantified by ELISA without complementary Western blot, immunohistochemistry, or transcriptional confirmation, and upstream mediators (PHD, Nrf2, mitochondrial intermediates) were inferred rather than measured. Finally, the functional consequence of the HIF-1α–caspase-3 signature — whether net hepatoprotective or potentially pro-apoptotic — was not assessed by histopathology or survival endpoints and warrants dedicated study.

4.13. Future directions

Future work should pursue four complementary lines. First, a graded-dose, multi-timepoint design would resolve the dose–response relationship and the temporal dynamics of HIF-1α stabilization. Second, orthogonal molecular validation — Western blotting, immunohistochemistry, and quantitative PCR of HIF-1α targets (VEGF, GLUT1, erythropoietin), Nrf2, PHD isoforms, and mitochondrial apoptotic intermediates — would convert the inferred pathway into a directly measured one. Third, histopathological and functional liver endpoints, together with markers of cellular senescence, would establish whether the caspase-3 increase reflects beneficial senescent-cell clearance or maladaptive apoptosis. Fourth, isolating standardized C-phycocyanin against the whole extract would clarify the contribution of individual phytochemicals and refine the structure–activity model that underpins rational herbal formulation.[7,26,27]

5. Conclusion

Oral *Spirulina platensis* extract stabilized hepatic HIF-1α by 60.0% and increased caspase-3 by 21.2% selectively in older Wistar rat liver, with very large effect sizes (Cohen's d of 5.37 and 2.13) and a strong HIF-1α–caspase-3 correlation (r = 0.879), while the autophagy adaptor p62/SQSTM1 remained unchanged. These results indicate that Spirulina engages the hepatic hypoxia–apoptosis axis in an age-dependent manner, most plausibly through C-phycocyanin-mediated redox modulation of the PHD–HIF-1α checkpoint, while sparing selective autophagy. The significant age × treatment interaction establishes age dependence as a quantitative feature of Spirulina action, while the moderated partial correlation frames the hypoxia–apoptosis coupling as associational. The age-restricted, coordinated molecular signature provides mechanistic, biochemical evidence supporting Spirulina as a complementary herbal hepatoprotective modality for the aging liver, consistent with the scope of evidence-based herbal medicine. Future work should define the dose–response relationship, confirm the pathway with orthogonal molecular methods and histopathology, measure upstream redox and PHD mediators, and evaluate functional hepatic outcomes to establish the therapeutic balance of this age-conditioned response.

Declarations

Use of Generative Artificial Intelligence. The authors declare that no generative artificial intelligence tool was used in the conception, design, conduct, analysis, or interpretation of this study, nor to generate any data, figure, or reference. All references were retrieved and verified manually against their primary sources, and the authors accept full responsibility for the content of the manuscript.

Author Contributions. R.P. conceived and designed the study, supervised the animal experiments and biochemical assays, performed the statistical analysis, and drafted the manuscript. F.C.I. contributed to the study design, extract standardization, and interpretation of the redox and hypoxia data. N.M. supervised the laboratory work, validated the ELISA procedures, and critically revised the manuscript. All authors reviewed, revised, and approved the final version and agree to be accountable for all aspects of the work.

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Conflict of Interest. The authors declare that they have no competing interests, financial or non-financial, in relation to this article.

Ethical Approval. All procedures involving animals were approved by the CMHC Ethics Committee (approval number 2024/120) and were conducted in accordance with institutional and international guidelines for the care and use of laboratory animals. Reporting follows the ARRIVE 2.0 guidelines.

Data Availability. The per-animal dataset and the analysis code supporting the findings of this study are available from the corresponding author on reasonable request.

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