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RESEARCH ARTICLE

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Age-dependent effects of *Spirulina platensis* on hepatic protein carbonylation in Wistar rats

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ABSTRACT

Background: stress increases with age and contributes to hepatic dysfunction through protein oxidation. Protein carbonyls are stable biomarkers of oxidative damage and can be used to assess age-related oxidative status. *Spirulina platensis* is a nutrient-rich cyanobacterium with established antioxidant properties.

Objective: To evaluate the effect of *Spirulina platensis* administration on hepatic protein carbonyl concentrations in male Wistar rats at different stages of young adulthood.

Methods: Male Wistar rats aged 8, 14, and 20 weeks received *Spirulina platensis* extract (200 mg/kg BW/day) orally for 29 days, corresponding to ages 12, 18, and 24 weeks at the time of sampling. Age-matched control groups received distilled water. Liver tissue was harvested on day 30. Protein carbonyl levels were quantified using the DNPH method, normalized to protein concentration, and analysed with Shapiro–Wilk tests and one-way ANOVA.

Results: In the control groups, carbonyl levels increased from 12 to 18 weeks (1.33-fold) and declined at 24 weeks (0.57-fold compared with 18 weeks), although not significantly ($p = 0.069$). Compared with age-matched controls, *Spirulina* administration increased carbonyl levels at 12 weeks (1.22-fold) but decreased them at 18 weeks (0.69-fold) and 24 weeks (0.92-fold); however, none of these differences reached statistical significance ($p = 0.069$).

Conclusion: *Spirulina platensis* demonstrated age-dependent effects on hepatic protein carbonylation, with potential antioxidant benefits in older young adult rats, although results require confirmation with larger sample sizes.

Keywords: protein carbonylation; oxidative stress; *Spirulina platensis*; aging; liver

Introduction

Aging is characterized by progressive physiological decline that increases susceptibility to disease, disability, and death, collectively termed senescence. Indonesia's population is rapidly aging, with the National Socioeconomic Survey (SUSENAS) 2019 reporting 25.7 million individuals aged 60 years and older, representing approximately 9.6% of the total population. This proportion is projected to increase substantially, reaching 40 million by 2035 and 1.5 billion globally by 2050 [1,2]. Consequently, the prevalence of age-related degenerative diseases, including hepatic disorders and diabetes, is expected to rise correspondingly [3].

Hepatic volume decreases by 20-40% during aging, accompanied by reduced blood flow and functional capacity. Age-related changes in liver function are closely associated with oxidative stress, which primarily targets hepatocytic proteins. Reactive oxygen and nitrogen species (RONS), produced by all aerobic cells, play crucial roles in aging and age-related diseases [4,5]. These species induce oxidative modifications in cellular macromolecules, including carbohydrates, lipids, proteins, and DNA, which serve as biomarkers of oxidative damage [2,4]. Protein carbonylation, the irreversible oxidative modification of proteins, represents a stable end-product of oxidative stress and serves as the first line of defense protecting against DNA oxidation [2,4]. Protein carbonyls accumulate with age due to post-translational modifications including glycation and glycoxidation [4]. When hepatic proteins are damaged by free radicals, carbonyl formation provides a quantifiable measure of oxidative injury [5].

Spirulina platensis, a filamentous cyanobacterium widely distributed in marine and freshwater environments, has attracted considerable attention as a functional food. Designated as a health food by the World Health Organization, *S. platensis* contains essential amino acids, proteins, polyunsaturated fatty acids, and potent antioxidant compounds including phycocyanin, β-carotene, and various carotenoids [6,7]. Its rich antioxidant profile and high nutritional value make it a promising candidate for preventing and treating diseases associated with oxidative stress, including cancer, diabetes, and inflammatory conditions [8].

Most previous studies examining oxidative stress in Wistar rats have utilized young animals aged 4-12 weeks, corresponding to the juvenile through adolescent developmental stages in humans (2-18 years) [9–11]. However, oxidative stress patterns and antioxidant responses may differ in older animals. Our study examined male Wistar rats aged 12, 18, and 24 weeks, corresponding to human emerging adulthood (18-25 years) and young adulthood (25-40 years) [12]. Male Wistar rats were selected based on their demonstrated physiological responsiveness to oxidative stress across various experimental models, including overtraining, intermittent fasting, and metabolic syndrome studies [9–11].

The present study aimed to investigate the age-dependent effects of *S. platensis* on hepatic protein carbonylation in young adult male Wistar rats (aged 12, 18, and 24 weeks). By examining carbonyl levels as a biomarker of oxidative protein damage across three age groups, we sought to characterize both age-related changes in hepatic oxidative stress and the potential protective effects of *Spirulina* supplementation. Understanding these dynamics may provide insights relevant to nutritional interventions for maintaining hepatic health during aging and inform future applications in human populations.

Methods

Animals and study design

Male Wistar rats aged 12, 18, and 24 weeks were obtained from an accredited animal facility. Animals were acclimatized for 7 days under controlled temperature (22 ± 2°C), 12-h light/dark cycle, and ad libitum access to food and water. For each age group, rats were randomly assigned to a control group or a *S. platensis* treatment group (n = 5 per group).

Spirulina platensis extract preparation

Ethanol extract of *S. platensis* was obtained from the Department of Chemistry, Faculty of Medicine, Universitas Indonesia. The *S. platensis* biomass was sourced from Balai Besar Perikanan Budidaya Air Payau (BBPBAP), Brackish Water Aquaculture Center, Jepara City, Central Java Province, Indonesia. Phytochemical characterization and thin-layer chromatography (TLC) analysis of the extract were performed as previously described [13,14]

Experimental design

This study was approved by the Ethics Commission of the Faculty of Medicine, Universitas Indonesia – RSUPN Dr. Cipto Mangunkusumo (approval number: KET-699/UN2.F1/ETIK. PPM.00.02/2020).

Thirty male Wistar rats were divided into six groups (n=5 per group) based on age and treatment (Table 1). Rats were obtained at 8, 14, and 20 weeks of age and received either *S. platensis* ethanol extract (200 mg/kg body weight) [15] or distilled water orally once daily for 29 days. The treatment period extended until the rats reached 12, 18, and 24 weeks of age, corresponding to human developmental stages of emerging adulthood (18-25 years) and young adulthood (25-40 years) [12].

On day 30, rats were euthanized, and liver tissue was immediately excised and stored at -80°C until analysis.

Sample preparation and homogenization

Liver tissue (100 mg) was placed in a 1.5 mL microcentrifuge tube and homogenized with 1 mL phosphate-buffered saline (PBS) using a mechanical homogenizer. Homogenization was performed in an

Table 1. Experimental groups and treatment protocol

Group	Initial age (weeks)	Final age (weeks)	Treatment	Duration (days)
Control 12	8	12	Distilled water	29
Spirulina 12	8	12	*S. platensis* 200 mg/kg BW	29
Control 18	14	18	Distilled water	29
Spirulina 18	14	18	*S. platensis* 200 mg/kg BW	29
Control 24	20	24	Distilled water	29
Spirulina 24	20	24	*S. platensis* 200 mg/kg BW	29

ice bath to maintain temperature at approximately 0°C. The homogenate was centrifuged at 3,500 rpm for 10 minutes at 4°C. The supernatant was collected and used for protein and carbonyl measurements.

Protein quantification

Total protein concentration was determined using the Warburg-Christian method [16]. Briefly, sample absorbance was measured at 280 nm using a spectrophotometer. Protein concentrations were calculated from a standard curve prepared with known protein concentrations.

Carbonyl level measurement

Protein carbonyl content was measured using the method described by Allen [17]. Protein carbonyl groups in the sample reacted with 2,4-dinitrophenylhydrazine (DNPH) to form stable hydrazone derivatives. These hydrazones are detectable spectrophotometrically at an absorbance of 370 nm. Carbonyl levels were obtained by dividing the absorbance value by the extinction coefficient (22.000 M^{-1}), then dividing by the protein concentration of each sample, and then multiplying by the dilution. Carbonyl level is expressed in units of nmol carbonyl per mg of protein

Data analysis

Data analysis was performed using Microsoft Excel and SPSS Statistics for Windows. Normal distribution of data was assessed using the Shapiro-Wilk test. Since data were normally distributed ($p > 0.05$), one-way analysis of variance (ANOVA) was used to compare groups. Post-hoc comparisons were conducted using Tukey's test. Results are presented as mean values, and differences were considered statistically significant at $p < 0.05$. All statistical tests were two-tailed.

Results

Carbonyl concentration with increasing age in control groups

The mean carbonyl concentration in control groups varied across age groups: 0.558 nmol/mg protein at 12 weeks, 0.743 nmol/mg protein at 18 weeks, and 0.423 nmol/mg protein at 24 weeks (Table 2). The 18-week control group exhibited the highest carbonyl concentration, while the 24-week group showed the lowest level.

Compared to the 12-week control group, carbonyl concentration in the 18-week control group increased 1.33-fold, whereas the 24-week control group showed a decrease to 0.76-fold of the 12-week level. The 24-week control group also demonstrated a reduction to 0.57-fold compared to the 18-week group. However, these age-related differences in carbonyl concentration were not statistically significant ($p = 0.069$, one-way ANOVA).

Effect of Spirulina platensis administration on carbonyl concentration

The highest carbonyl level in the *Spirulina*-treated groups was observed in the 12-week group (0.678 nmol/mg protein), while the lowest was

Table 2. Carbonyl concentration in liver tissue of control groups at different ages

Age group	Carbonyl concentration (nmol/mg protein)	Ratio compared to 12 weeks	Ratio compared to 18 weeks	p-value*
12 weeks	0.558	1.00	0.75	0.069
18 weeks	0.743	1.33	1.00	
24 weeks	0.423	0.76	0.57	

*One-way ANOVA

Table 3. Effect of *Spirulina platensis* administration on liver carbonyl concentration across age groups

Age group	Control (nmol/mg protein)	*Spirulina* (nmol/mg protein)	Ratio (*Spirulina*/Control)	Percentage change	p-value*
12 weeks	0.558	0.678	1.22	+22%	0.069
18 weeks	0.743	0.510	0.69	-31%	
24 weeks	0.423	0.391	0.92	-8%	

*One-way ANOVA

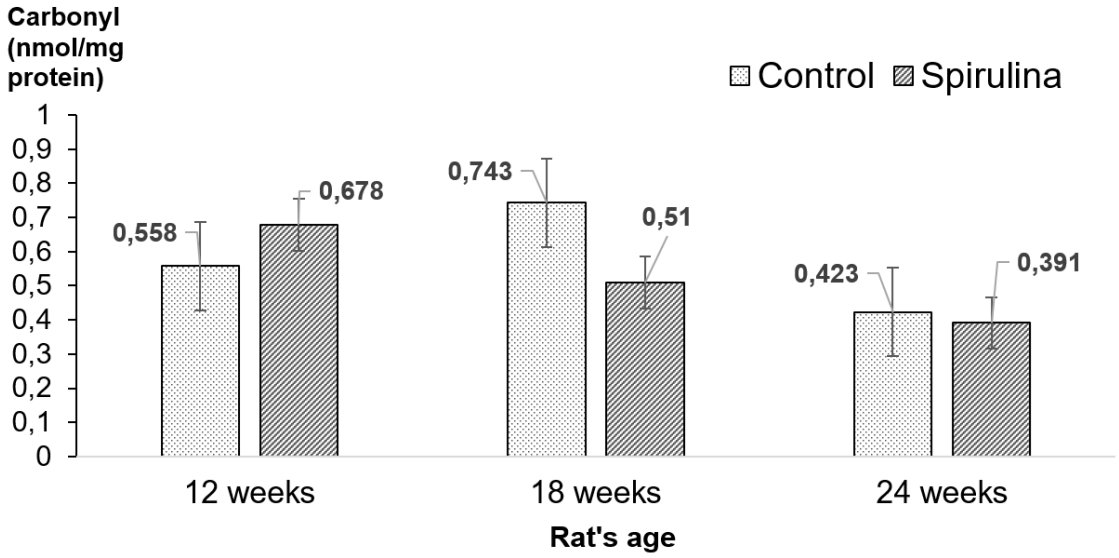

Figure 1. Carbonyl concentration in liver tissue of male Wistar rats treated with *Spirulina platensis* compared to control groups across different ages. Error bars represent standard error. P = 0.069 (One-way ANOVA)

in the 24-week group (0.391 nmol/mg protein) (Table 3, Figure 1).

In the 12-week group, *S. platensis* administration resulted in a carbonyl concentration of 1.22-fold compared to the age-matched control group, representing a 22% increase. Conversely, the 18-week *Spirulina*-treated group showed a reduction to 0.69-fold (31% decrease) compared to controls. The 24-week *Spirulina*-treated group demonstrated a carbonyl concentration of 0.92-fold (8% decrease) relative to the control group. Despite these observed trends, none of the differences between *Spirulina*-treated and control groups reached statistical significance (p = 0.069, one-way ANOVA).

Data are presented as mean values. Normality testing using Shapiro-Wilk showed normal distribution in both control (p = 0.924) and *Spirulina* groups (p = 0.858).

Discussion

This study investigated the effects of *S. platensis* administration on hepatic protein carbonylation in male Wistar rats of different ages. Our findings revealed a non-linear pattern of carbonyl accumulation with age in control animals, with peak levels observed at 18 weeks followed by a decline at 24 weeks. Although *S. platensis* administration showed a trend toward reducing carbonyl levels in older rats (18 and 24 weeks), these changes did not reach statistical significance. Interestingly, the youngest age group (12 weeks) exhibited a slight increase in carbonyl levels following *Spirulina* treatment. These results suggest potential age-dependent effects of *S. platensis* on hepatic oxidative stress, though the lack of statistical significance indicates the need for further investigation with larger sample sizes and optimized treatment protocols.

The non-linear pattern of carbonyl concentration observed in our control groups, with levels increasing from 12 to 18 weeks and then decreasing at 24 weeks, diverges from the conventional understanding that oxidative damage accumulates progressively with age. This unexpected finding may reflect complex adaptive mechanisms in young adult rats. The peak at 18 weeks could indicate a period of heightened metabolic activity or oxidative stress during the transition from emerging to young adulthood. The subsequent decline at 24 weeks might represent a compensatory response through enhanced cellular repair mechanisms or improved antioxidant defenses. However, it is important to note that our study examined relatively young rats (12-24 weeks, equivalent to 18-40 human years), whereas most aging research focuses on much older animals. Kolawole et al. (2019) demonstrated progressive increases in carbonylated proteins with age in brain, heart, and kidney tissues of Wistar rats aged 25-90 days, though their study did not extend to the age range examined in our research [18]. The differences in tissue types and age ranges may explain the discrepant findings.

Previous work from our research group provides context for these carbonyl findings. Paramita et al. (2024) reported that hepatic catalase specific activity declined progressively with age in the same rat cohorts, with the highest activity at 12 weeks (0.028 U/mg protein) and lowest at 24 weeks (0.009 U/mg protein) [19]. This decline in catalase activity would be expected to result in increased oxidative stress and protein carbonylation in older animals. However, the non-linear carbonyl pattern we observed suggests that other antioxidant systems or adaptive mechanisms may compensate for reduced catalase activity. Additionally, the same research group found that malondialdehyde (MDA), a marker of lipid peroxidation, decreased after *S. platensis* administration across all age groups, with the most pronounced effect at 18 weeks [19]. The parallel reduction in both lipid peroxidation (MDA) and protein oxidation (carbonyls) at 18 weeks following *Spirulina* treatment suggests a comprehensive antioxidant effect, though the magnitude of response differs between oxidative markers.

Luceri et al. (2018) reported that increases in circulating reactive oxygen species (ROS) and protein carbonyl content occur as early as middle age, with oxidative DNA damage becoming particularly noticeable in middle-aged animals even under normal physiological conditions [5]. Their findings support the concept that oxidative stress biomarkers can fluctuate substantially during the aging process rather than increasing linearly. Protein carbonylation serves as a hallmark of oxidative damage and an indicator of oxidative stress in aging and age-related disorders [20]. Yang et al. demonstrated that serum alanine transaminase (ALT) and nitrotyrosine protein expression, both markers of oxidative stress and cellular damage, increased significantly in 24-month-old Wistar rats compared to younger groups [22]. The discrepancy between Yang's findings and our results may be attributed to the substantial age difference in the animals studied; our oldest rats were 24 weeks (approximately 6 months), while Yang's aged rats were 24 months (approximately 2 years in rat age).

The differential response to *S. platensis* administration across age groups warrants careful interpretation. The reduction in carbonyl levels

observed in the 18-week and 24-week *Spirulina*-treated groups aligns with the established antioxidant properties of *Spirulina* species. Neyrinck et al. found that *Spirulina* diet significantly downregulated hepatic inflammatory and oxidative biomarkers in 24-month-old mice (equivalent to 96 weeks) compared to controls [21]. Although their study used older animals, longer treatment duration (42 days), and dietary supplementation (5% powder in standard diet) rather than oral gavage, their findings support the antioxidant efficacy of *Spirulina* in aged liver tissue. The more pronounced carbonyl reduction in our 18-week group may indicate that this age represents an optimal window for *Spirulina*'s antioxidant intervention, possibly due to the peak oxidative stress observed at this age in control animals.

The unexpected increase in carbonyl levels in the 12-week *Spirulina*-treated group challenges our initial hypothesis and requires mechanistic consideration. In younger animals with inherently lower baseline oxidative stress, *Spirulina* administration may trigger a hormetic response or temporarily alter the balance of pro-oxidant and antioxidant systems. Alternatively, the bioactive compounds in *Spirulina*, particularly phycocyanin and β-carotene, may require a threshold level of oxidative stress to exert protective effects. When baseline oxidative damage is minimal, as in the 12-week rats, these compounds might not confer the same protective benefits or could even have pro-oxidant effects under certain conditions. This age-dependent response has implications for the clinical application of *Spirulina* supplementation and suggests that benefits may be most pronounced in populations experiencing elevated oxidative stress.

The antioxidant mechanisms of *Spirulina* species have been extensively characterized. Aissaoui et al. demonstrated that *S. platensis* administration significantly increased total antioxidant status (43% increase, $p < 0.01$), glutathione reductase (16% increase, $p < 0.01$), superoxide dismutase (48% increase, $p < 0.001$), and glutathione peroxidase (37% increase, $p < 0.001$) [22]. These effects are attributed primarily to phycocyanin, phycocyanobilin, and β-carotene, which actively prevent lipid peroxidation, scavenge free radicals, and reduce inflammation [23,24]. The comprehensive upregulation of multiple antioxidant enzymes explains *Spirulina*'s broad protective effects across various organ systems, including the nervous system, liver, intestine, cardiovascular system, and skeletal muscle [3,8,25]. The relatively short administration period in our study (29 days) may not have been sufficient to fully activate these enzymatic antioxidant systems, particularly in younger animals with already robust endogenous defenses.

Spirulina administration reduced liver carbonyl levels by 0.686-fold at 18 weeks and 0.924-fold at 24 weeks compared to controls, suggesting that *Spirulina*'s antioxidative effect is most effective during mid-age stages. The same study reported that *Spirulina* administration reduced catalase specific activity across all age groups, with the most pronounced reduction at 18 weeks (0.55-fold compared to control) [26]. This paradoxical reduction in catalase activity alongside reduced oxidative stress markers suggests that *Spirulina* may influence antioxidant enzyme regulation in complex, dose-dependent ways, potentially through feedback mechanisms that reduce the need for certain endogenous antioxidant enzymes when exogenous antioxidants are abundant.

The lack of statistical significance in our findings must be interpreted cautiously. Nakata et al. similarly reported no statistically significant differences in protein carbonyl levels between *Spirulina* powder intervention and control groups in *Mus musculus* mice, attributing the insignificant results to short exposure duration [27]. Our study shares similar limitations: a relatively small sample size (n=5 per group), short treatment duration (29 days), and examination of young rats with potentially low baseline oxidative stress. Power analysis would be valuable to determine the sample size required to detect significant differences given the observed effect sizes. Despite the lack of statistical significance, the consistent direction of change (reduction) in the two older age groups and the magnitude of reduction (31% at 18 weeks) suggest biological relevance that merits further investigation. Future studies should consider longer treatment durations,

larger sample sizes, and inclusion of aged rats (18-24 months) where oxidative stress accumulation is more pronounced and *Spirulina*'s protective effects might be more evident.

A notable strength of this study is its novelty in examining the effects of *S. platensis* on liver carbonyl levels across multiple age groups in young adult Wistar rats, an area not previously explored in the literature. However, several limitations must be acknowledged. First, we examined only a single biomarker of protein oxidation (carbonyl); a more comprehensive assessment including multiple oxidative stress markers (MDA, glutathione status, antioxidant enzyme activities) would provide a fuller picture of *Spirulina*'s effects. Second, we did not measure *Spirulina*'s bioactive compounds in plasma or liver tissue to confirm absorption and bioavailability. Third, the study design did not include older rats (beyond 24 weeks) where age-related oxidative damage would be more pronounced. Fourth, we used ethanol extract administered by oral gavage, which may have different bioavailability compared to whole *Spirulina* incorporated into diet. Finally, the study examined only male rats; sex differences in oxidative stress responses and *Spirulina* efficacy should be investigated. Future research should address these limitations by examining a broader range of ages including aged rats, incorporating multiple oxidative stress biomarkers, measuring bioactive compound concentrations, comparing different *Spirulina* formulations and doses, extending treatment duration, including both sexes, and conducting mechanistic studies to elucidate the molecular pathways underlying age-dependent responses to *Spirulina* supplementation.

Conclusion

This study shows that hepatic protein carbonyl levels exhibit age-dependent variation in male Wistar rats, with a transitional rise at 18 weeks and a decline at 24 weeks. Spirulina platensis supplementation modulated these patterns, attenuating carbonyl accumulation in older rats despite no statistically significant differences. These trends indicate that Spirulina may exert greater antioxidant benefit when baseline oxidative stress is elevated, particularly in early adulthood. Further studies with larger sample sizes and additional redox biomarkers are required to confirm these age-specific effects and clarify Spirulina's mechanistic role in hepatic oxidative regulation.

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Declaration of interest

The authors declare no conflicts of interest with any private, public, or academic entities related to the content of this manuscript.

Author contributions

RP: Conceptualization, Methodology, Investigation, Resources, Funding acquisition, Project administration, Writing – original draft, Writing – review & editing. TRZ: Investigation, Formal analysis, Data curation, Validation, Writing – original draft, Writing – review & editing. All authors have read and approved the final manuscript.

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References

1. Statistik dan Portal Satudata Jakarta - Data Terbuka DKI Jakarta. 2019. Available: https://statistik.jakarta.go.id/statisik-penduduk-lanjut-usia-di-dki-jakarta-tahun-2019/
2. Pusat Data dan Informasi Kementrian Kesehatan RI. Infodatin lansia 2022. 2022.
3. Wu Q, Liu L, Miron A, Klímová B, Wan D, Kuča K. The antioxidant, immunomodulatory, and anti-inflammatory activities of Spirulina: an overview. Arch Toxicol. 2016;90: 1817-1840. https://doi.org/10.1007/s00204-016-1744-5
4. Li S, Tan H-Y, Wang N, Zhang Z-J, Lao L, Wong C-W, et al. The Role of Oxidative Stress and Antioxidants in Liver Diseases. Int J Mol Sci. 2015;16: 26087-26124. https://doi.org/10.3390/ijms161125942
5. Luceri C, Bigagli E, Femia A Pietro, Caderni G, Giovannelli L, Lodovici M. Aging related changes in circulating reactive oxygen species (ROS) and protein carbonyls are indicative of liver oxidative injury. Toxicol Rep. 2018;5: 141-145. https://doi.org/10.1016/j.toxrep.2017.12.017
6. Buwono NR, Nurhasanah RQ. Studi Pertumbuhan Populasi Spirulina sp. pada Skala Kultur yang Berbeda *[Study of Spirulina sp. Population Growth in The Different Culture Scale]. Jurnal Ilmiah Perikanan dan Kelautan. 2018;10: 26.* https://doi.org/10.20473/jipk.v10i1.8516
7. Seyidoglu N, Inan S, Aydin C. A Prominent Superfood: Spirulina platensis. Superfood and Functional Food - The Development of Superfoods and Their Roles as Medicine. 2017. https://doi.org/10.5772/66118
8. Gabr GA, El-Sayed SM, Hikal MS. Antioxidant Activities of Phycocyanin: A Bioactive Compound from Spirulina platensis. J Pharm Res Int. 2020; 73-85. https://doi.org/10.9734/jpri/2020/v32i230407
9. Nyandra M, kartiko bambang, Arunngam P, pangkahila alex, Siswanto F. Overtraining induces oxidative stress-mediated renal damage in male Wistar rats. Transylvanian Review. 2018;26.
10. Nurmasitoh T, Utami SY, Kusumawardani E, Najmuddin AA, Fidianingsih I. Intermittent fasting decreases oxidative stress parameters in Wistar rats (Rattus norvegicus). Universa Medicina. 2018;37: 31. https://doi.org/10.18051/UnivMed.2018.v37.31-38
11. Di Majo D, Sardo P, Giglia G, Di Liberto V, Zummo FP, Zizzo MG, et al. Correlation of Metabolic Syndrome with Redox Homeostasis Biomarkers: Evidence from High-Fat Diet Model in Wistar Rats. Antioxidants. 2022;12: 89. https://doi.org/10.3390/antiox12010089
12. Ghasemi A, Jeddi S, Kashfi K. The laboratory rat: Age and body weight matter. EXCLI J. 2021; 1431-1445.
13. Prijanti AR, Nurhayati RW, Iswanti FC, Khoiriyah Z, Paramita R, Fadilah F. Java Sea Spirulina platensis chemical analysis and its protective ability against H2O2-exposed umbilical cord mesenchymal stem cells according to CD73, CD90, and CD105, viability, and HIF-1 alpha docking. J Appl Pharm Sci. 2022. https://doi.org/10.7324/JAPS.2022.120908
14. Paramita R, Purba HHS, Prijanti AR, Iswanti FC. Supplementation Impact of Spirulina platensis Ethanol Extract on Inflammatory Homeostasis Modulation of Rat Spleen at Different Ages. Hayati. 2024;31: 1231-1242. https://doi.org/10.4308/hjb.31.6.1231-1242
15. Pérez-Juárez A, Aguilar-Faisal JL, Posadas-Mondragón A, Santiago-Cruz JA, Barrientos-Alvarado C, Mojica-Villegas MA, et al. Effect of Spirulina (Formerly Arthrospira) Maxima against Ethanol-Induced Damage in Rat Liver. Applied Sciences. 2022;12: 8626. https://doi.org/10.3390/app12178626
16. Mudjihartini N, Prasetya SI, Sadikin M. Plasma Protein Profile of Lactating Women from Two Primary Health Centers in Jakarta, Indonesia. Rep Biochem Mol Biol. 2022;11: 209-215. https://doi.org/10.61186/rbmb.11.2.209
17. Dewi S, Sadikin M, Mulyawan W. Oxidative stress in the heart of rats exposed to acute intermittent hypobaric hypoxia. The Ukrainian Biochemical Journal. 2021;93: 68-74. https://doi.org/10.15407/ubj93.03.068
18. Kolawole TA, Ilochi ON, Oluwatayo BO, Chuemere AN, Dapper D V. Tissue Protein Carbonylation in Aging: A Strategic Analysis of Age-Related Protein Modification. J Adv Med Pharm Sci. 2019; 1-5. https://doi.org/10.9734/jamps/2019/v20i330111
19. Paramita R. Spirulina platensis administration decreases malondialdehyde level of rat's liver at various ages. Oral presentation at: International Conference of the Indonesian Society for Biochemistry and Molecular Biology (ICISBMB 2024); 2024 Jun 6; Purwokerto, Indonesia. Unpublished conference proceeding.
20. Akagawa M. Protein carbonylation: molecular mechanisms, biological implications, and analytical approaches. Free Radic Res. 2020;55: 1-37. https://doi.org/10.1080/10715762.2020.1851027
21. Neyrinck A, Taminiau B, Walgrave H, Daube G, Cani P, Bindels L, et al. Spirulina Protects against Hepatic Inflammation in Aging: An Effect Related to the Modulation of the Gut Microbiota? Nutrients. 2017;9: 633. https://doi.org/10.3390/nu9060633
22. Hoseini SM, Khosravi-Darani K, Mozafari MR. Nutritional and Medical Applications of Spirulina Microalgae. Mini-Reviews in Medicinal Chemistry. 2013;13: 1231-1237. https://doi.org/10.2174/1389557511313080009
23. Abdelkhalek NKM, Ghazy EW, Abdel-Daim MM. Pharmacodynamic interaction of Spirulina platensis and deltamethrin in freshwater fish Nile tilapia, Oreochromis niloticus: impact on lipid peroxidation and oxidative stress. Environmental Science and Pollution Research. 2014;22: 3023-3031. https://doi.org/10.1007/s11356-014-3578-0
24. K.G. MG, K US, R S, G.A. R. Supercritical CO2 extraction of functional compounds from Spirulina and their biological activity. J Food Sci Technol. 2014. https://doi.org/10.1007/s13197-014-1426-3

25. Ismail Md, Hossain MdF, Tanu AR, Shekhar HU. Effect of Spirulina Intervention on Oxidative Stress, Antioxidant Status, and Lipid Profile in Chronic Obstructive Pulmonary Disease Patients. Biomed Res Int. 2015;2015: 1-7. https://doi.org/10.1155/2015/486120

26. Paramita NR, Mumtaz A. Effect of Spirulina platensis administration on liver catalase specific activities of various ages Wistar Rats. World Journal of Biology Pharmacy and Health Sciences. 2025;22: 283-289. https://doi.org/10.30574/wjbphs.2025.22.2.0477

27. Katsuura S, Imamura T, Bando N, Yamanishi R. β-Carotene and β-cryptoxanthin but not lutein evoke redox and immune changes in RAW264 murine macrophages. Mol Nutr Food Res. 2009;53: 1396-1405. https://doi.org/10.1002/mnfr.200800566