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Age-dependent effects of *Spirulina platensis* ethanol extract supplementation on brain malondialdehyde levels in young adult Wistar rats

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Abstract

Background: Oxidative stress, reflected by elevated malondialdehyde (MDA) levels, is a key mediator of brain aging and neurodegeneration. *Spirulina platensis*, rich in phycocyanin and other antioxidants, is hypothesized to reduce lipid peroxidation and attenuate brain oxidative stress.

Objective: To investigate the age-dependent effects of *Spirulina platensis* ethanol extract supplementation on brain MDA levels in young adult male Wistar rats aged 12, 18, and 24 weeks.

Methods: Thirty male Wistar rats were divided into six groups (n=5 per group): three control groups receiving distilled water and three treatment groups receiving 200 mg/kgBW of *Spirulina platensis* ethanol extract by oral gavage for 29 days, starting at 8, 14, and 20 weeks of age, respectively, and sacrificed at 12, 18, and 24 weeks. Brain tissue MDA levels were measured using the Wills method (thiobarbituric acid assay) with UV-Vis spectrophotometry at 530 nm. Data were analyzed using one-way ANOVA followed by post-hoc Tukey test, with significance set at $p<0.05$.

Results: In control groups, brain MDA levels showed a declining trend with age: 1.702±0.185, 1.286±0.272, and 1.108±0.306 nmol/mL at 12, 18, and 24 weeks, respectively, with a significant difference between 12-week and 24-week groups ($p=0.037$). In Spirulina-treated groups, MDA levels were 1.835±0.361, 0.992±0.286, and 1.214±0.311 nmol/mL at 12, 18, and 24 weeks, respectively. No statistically significant differences were observed between Spirulina-treated and control groups at any corresponding age ($p=0.978$, $p=0.610$, and $p=0.992$ at 12, 18, and 24 weeks, respectively).

Conclusion: *Spirulina platensis* ethanol extract at 200 mg/kgBW does not significantly reduce brain MDA levels in young adult Wistar rats across all age groups. Brain MDA levels tend to decrease with age in young adult rats, independent of Spirulina supplementation.

Keywords: *Spirulina platensis*; Malondialdehyde; Brain Oxidative Stress; Lipid Peroxidation; Wistar Rats; Aging

1. Introduction

The aging process is defined as the gradual accumulation of biological changes over time, resulting in progressive deterioration of physiological function and heightened vulnerability to disease [1]. At the molecular level, aging involves DNA instability, telomere shortening, mitochondrial dysfunction, and the accumulation of oxidatively damaged macromolecules [2,3]. Among the biochemical mechanisms underlying aging, oxidative stress — arising from an

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imbalance between reactive oxygen species (ROS) production and antioxidant defense capacity — plays a central role in both the aging process itself and the pathogenesis of age-related diseases [3,4].

The brain is uniquely vulnerable to oxidative stress owing to its high metabolic rate, abundant polyunsaturated fatty acid (PUFA) content, and comparatively limited antioxidant defenses [5]. Oxidative damage in the brain disrupts cellular signaling pathways, impairs neurotransmitter function, and promotes the aggregation of toxic protein species, thereby increasing the risk of neurodegenerative disorders such as Alzheimer's and Parkinson's diseases [6,7]. Reactive oxygen species accumulate in the brain over time, exacerbating oxidative stress and neuronal damage [4].

Malondialdehyde (MDA) is a well-established biomarker of lipid peroxidation and oxidative stress. It is generated when free radicals take hydrogen atoms from PUFAs, initiating a chain reaction that produces lipid hydroperoxides, which subsequently decompose into MDA and other reactive aldehydes [8]. MDA is mutagenic and cytotoxic, capable of forming DNA-protein crosslinks and adducts, and its elevated levels have been associated with neurodegenerative diseases, cardiovascular disorders, and cancer [8,9]. Measuring brain MDA levels thus provides a reliable index of oxidative stress in neural tissue.

Spirulina platensis, a blue-green microalga (cyanobacterium), has attracted considerable scientific interest as a natural antioxidant supplement. Its antioxidant capacity derives primarily from phycocyanin — a biliprotein pigment that scavenges peroxyl radicals and donates electrons to free radical species — as well as from β-carotene, tocopherols, polyphenols, and enzymatic antioxidants including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) [10,11]. Several preclinical studies have demonstrated that Spirulina supplementation reduces MDA levels and attenuates oxidative stress in various tissues and disease models [12,13]. However, evidence specifically examining the effect of *Spirulina platensis* on brain MDA levels across different age groups remains limited.

This study was therefore designed to investigate the age-dependent effects of *Spirulina platensis* ethanol extract supplementation on brain MDA levels in young adult male Wistar rats at 12, 18, and 24 weeks of age. We hypothesized that *Spirulina platensis* administration would lead to a significant reduction in brain MDA levels across all age groups compared to controls.

2. Materials and methods

2.1. Ethical Approval

This study was conducted in accordance with ethical standards for animal research. Ethical clearance was obtained from the Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia, under reference number KET-699/UN2.F1/ETIK/PPM.00.02/2020.

2.2. Study Design and Animals

This in vivo experimental study used stored brain tissue from male Wistar rats. Thirty male Wistar rats were obtained from the Health Research and Development Agency (PUSLITBANGKES), Ministry of Health, Republic of Indonesia. Animals were housed under standard laboratory conditions (12-hour light/dark cycle, ad libitum access to food and water, controlled temperature 22–25°C). Rats were divided into six groups (n=5 per group) based on age at sacrifice and treatment (Table 1).

Table 1 Experimental groups, treatment periods, and interventions (n=5 per group)

Group	Age at Sacrifice (weeks)	Treatment Period	Treatment
K12	12	Week 8 → Week 12	Distilled water (control)
S12	12	Week 8 → Week 12	*Spirulina platensis* 200 mg/kgBW/day
K18	18	Week 14 → Week 18	Distilled water (control)
S18	18	Week 14 → Week 18	*Spirulina platensis* 200 mg/kgBW/day
K24	24	Week 20 → Week 24	Distilled water (control)
S24	24	Week 20 → Week 24	*Spirulina platensis* 200 mg/kgBW/day

Inclusion criteria: stored male Wistar rat brain tissues from animals receiving either *Spirulina platensis* (200 mg/kgBW/day) or distilled water for 29 days. Exclusion criteria: brain tissues from unhealthy or female rats. Sample size was determined using the Federer formula: (n−1)(t−1) > 15, yielding a minimum of 4 rats per group; 5 rats per group were used to increase statistical power.

2.3. *Spirulina platensis* Extract Preparation

The ethanol extract of *Spirulina platensis* was obtained from Spirulina cultivated in the Java Sea and produced by the Department of Chemistry, Faculty of Medicine, Universitas Indonesia. The extract was administered daily by oral gavage at a dose of 200 mg/kgBW dissolved in distilled water for 29 consecutive days.

2.4. Brain Tissue Homogenization

Stored Wistar rat brain tissues were retrieved from a deep-freezer at −80°C. For each sample, 100 mg of brain tissue was placed in a 1.5 mL microcentrifuge tube and diluted with 1 mL of phosphate-buffered saline (PBS, 0.05 M, pH 7.0). Samples were homogenized using a tissue homogenizer, then centrifuged at 3,500 rpm for 10 minutes at 4°C. The resulting supernatant was collected and stored at −20°C until MDA measurement.

2.5. MDA Level Measurement (Wills Method)

Brain MDA levels were measured using the thiobarbituric acid (TBA) assay as described by Wills [14]. Briefly, 400 µL of brain homogenate supernatant was transferred to a microtube. For protein precipitation, 200 µL of 20% trichloroacetic acid (TCA) solution (20 mg TCA dissolved in 100 mL distilled water) was added, vortexed, and centrifuged at 5,000 rpm for 10 minutes. The supernatant was collected and 400 µL of 0.67% TBA solution (0.67 mg TBA dissolved in 100 mL distilled water) was added to each reaction tube. Tubes were incubated in a water bath at 96–100°C for 10 minutes to generate a pink MDA-TBA chromogen, then cooled to room temperature. Absorbance was measured at 530 nm using a UV-Vis spectrophotometer. All measurements were performed in duplicate.

A standard curve was constructed using 1,1,3,3-tetraethoxypropane (TEP) as the MDA precursor at concentrations of 0.3125, 0.625, 1.25, 2.5, and 5.0 nmol/mL. The standard curve equation was: $y = 0.0762x + 0.0037$ ($R^2 = 0.9991$), where x is the absorbance value and y is the MDA concentration in nmol/mL.

2.6. Statistical Analysis

Data are expressed as mean ± standard deviation (SD). Normality of data distribution was assessed using the Shapiro-Wilk test (all groups: $p > 0.05$). Differences among groups were analyzed using one-way ANOVA, followed by post-hoc Tukey's Honestly Significant Difference (HSD) test for multiple comparisons. All analyses were performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Statistical significance was set at $p < 0.05$.

3. Results and discussion

3.1. MDA Standard Curve

The MDA standard curve was constructed using TEP at concentrations of 0.3125, 0.625, 1.25, 2.5, and 5.0 nmol/mL. The resulting linear equation was $y = 0.0762x + 0.0037$ with a coefficient of determination $R^2 = 0.9991$, confirming excellent linearity across the measured concentration range and validating the reliability of the TBA assay for quantifying MDA in brain tissue homogenates.

3.2. Brain MDA Levels Across Age Groups

Brain MDA concentrations for all six groups are summarized in Table 2 and Figure 1. In the control groups (distilled water), a declining trend in MDA levels was observed with increasing age: 1.702 ± 0.185 nmol/mL at 12 weeks (K12), 1.286 ± 0.272 nmol/mL at 18 weeks (K18), and 1.108 ± 0.306 nmol/mL at 24 weeks (K24). In the Spirulina-treated groups, MDA levels were 1.835 ± 0.361 nmol/mL at 12 weeks (S12), 0.992 ± 0.286 nmol/mL at 18 weeks (S18), and 1.214 ± 0.311 nmol/mL at 24 weeks (S24).

Table 2 Brain MDA levels (nmol/mL) in control and *Spirulina platensis*-treated Wistar rats

Group	Age at Sacrifice (weeks)	Treatment	MDA Level (nmol/mL, Mean ± SD)
K12	12	Distilled water	1.702 ± 0.185
S12	12	*Spirulina platensis* 200 mg/kgBW	1.835 ± 0.361
K18	18	Distilled water	1.286 ± 0.272
S18	18	*Spirulina platensis* 200 mg/kgBW	0.992 ± 0.286
K24	24	Distilled water	1.108 ± 0.306
S24	24	*Spirulina platensis* 200 mg/kgBW	1.214 ± 0.311

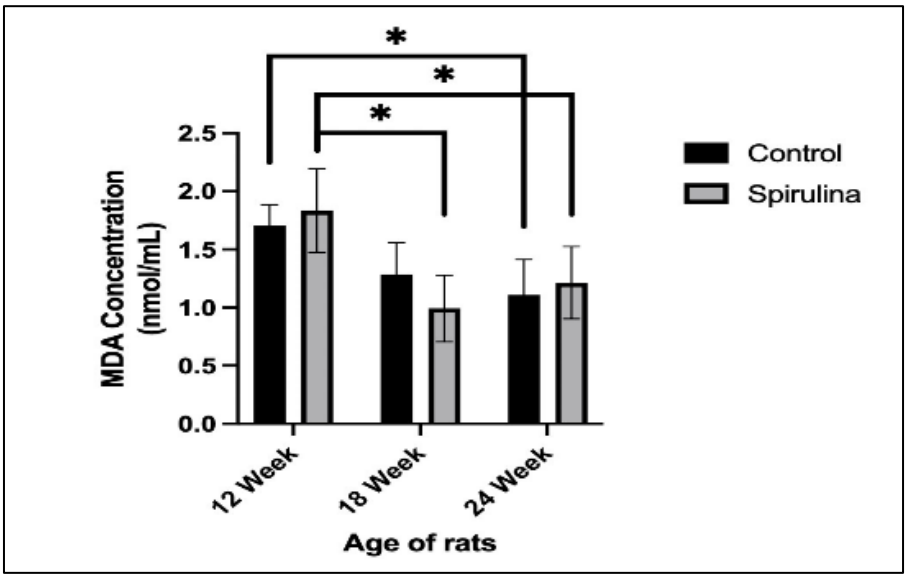

Figure 1 MDA level in 12, 18, and 24 Weeks old rats treated with distilled water (Control) and *Spirulina platensis*

3.3. Statistical Analysis

The Shapiro-Wilk normality test confirmed that all data sets followed a normal distribution ($p > 0.05$). One-way ANOVA revealed a statistically significant overall difference in MDA levels among the six groups ($p = 0.0001$). Post-hoc Tukey's HSD analysis identified the following significant pairwise comparisons (Table 3):

Table 3 Post-hoc Tukey HSD results for pairwise comparisons of brain MDA levels (*$p < 0.05$)

Comparison	Mean Difference (nmol/mL)	p-value	Significance
K12 vs. K18	0.416	0.251	Not significant
K12 vs. K24	0.594	0.037	Significant*
K18 vs. K24	0.178	0.923	Not significant
S12 vs. S18	0.843	0.020	Significant*
S12 vs. S24	0.621	0.027	Significant*
S18 vs. S24	0.222	0.832	Not significant
K12 vs. S12	−0.133	0.978	Not significant
K18 vs. S18	0.294	0.610	Not significant
K24 vs. S24	−0.106	0.992	Not significant

3.4. Age-Related Changes in Brain MDA Levels

The control groups demonstrated a statistically significant decline in brain MDA levels between 12 and 24 weeks ($p = 0.037$), with a 0.65-fold reduction in MDA concentration. Although the differences between 12 and 18 weeks ($p = 0.251$) and between 18 and 24 weeks ($p = 0.923$) were not statistically significant, the overall declining trend is consistent and biologically meaningful.

This finding must be contextualized within the specific age range examined (12–24 weeks), which corresponds to the young adulthood phase in rats — equivalent to approximately 18–40 years in humans [15]. During this developmental period, the rat brain is still undergoing maturation, with active adult neurogenesis occurring in regions such as the hippocampal dentate gyrus and lateral subventricular zone [16]. The presence of robust endogenous antioxidant capacity and ongoing neurogenesis during young adulthood may account for the declining MDA trend observed, as newly generated neurons may contribute to cellular renewal and reduced oxidative burden.

These findings are consistent with those of Mooradian et al. [17], who reported that tissue MDA levels peak at approximately 12 months of age in rats and subsequently decline, suggesting that MDA accumulation does not linearly correlate with lipid peroxidation across all life stages. Similarly, Dirgahayu et al. [18] reported lower MDA levels in older control rats compared to younger ones. Cordiano et al. [19] and Gil et al. [20] further support the concept that MDA dynamics during young adulthood differ substantially from those in aged organisms.

From a mechanistic perspective, the declining MDA trend in young adult rats may be attributed to: (1) upregulation of endogenous antioxidant enzyme activity (SOD, CAT, GPx) during the transition from adolescence to adulthood; (2) membrane lipid remodeling, whereby the proportion of highly peroxidation-susceptible PUFAs may decrease relative to more saturated lipids during brain maturation; and (3) active adult neurogenesis in the hippocampal dentate gyrus, which may contribute to cellular renewal and reduced overall oxidative burden [21]. Furthermore, studies with shorter age intervals between groups tend to show more variable MDA trends, as shorter intervals may not capture the cumulative changes in oxidative stress that become apparent over longer time periods [20].

3.5. Effect of *Spirulina platensis* on Brain MDA Levels

The Spirulina-treated groups showed variable MDA responses across age groups. Compared to age-matched controls, Spirulina supplementation resulted in a non-significant increase in MDA at 12 weeks (+0.133 nmol/mL; $p = 0.978$) and 24 weeks (+0.106 nmol/mL; $p = 0.992$), while producing a non-significant decrease at 18 weeks (−0.294 nmol/mL; $p = 0.610$). None of these differences reached statistical significance, indicating that *Spirulina platensis* at 200 mg/kgBW did not significantly alter brain MDA levels in healthy young adult Wistar rats.

These findings are consistent with a systematic review by Maddiboyina et al. [22], which highlighted that the antioxidant efficacy of Spirulina is highly context-dependent, varying with dose, duration, species, tissue type, and health status of the experimental subjects. Chaouachi et al. [23] demonstrated significant MDA reduction by Spirulina in exercise-induced oxidative stress models, while Hwang et al. [24] showed neuroprotective effects in senescence-accelerated mice. The discrepancy with our findings underscores the importance of baseline oxidative stress levels: in healthy young adult rats with intact endogenous antioxidant systems, additional exogenous antioxidant supplementation may not produce a detectable incremental reduction in MDA, consistent with the concept of antioxidant redundancy in healthy tissues [27].

The absence of a significant antioxidant effect of Spirulina in this study may be explained by several biochemical mechanisms. First, the dose of 200 mg/kgBW may be insufficient to produce a measurable antioxidant effect in the brain. Prior research has suggested that doses of approximately 1,000 mg/kgBW may be required to significantly reduce MDA levels in rat brain tissue [24]. Second, *Spirulina platensis* contains iron, which can participate in Fenton-type reactions ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH\bullet + OH^-$), generating hydroxyl radicals and potentially exerting a pro-oxidant effect at certain doses or in specific tissue environments [3,25]. Third, the high PUFA content of Spirulina — particularly gamma-linolenic acid — may itself be susceptible to peroxidation upon absorption, potentially contributing to transient increases in systemic lipid peroxidation products [26].

The significant differences observed within the Spirulina-treated groups between 12 and 18 weeks ($p = 0.020$) and between 12 and 24 weeks ($p = 0.027$) suggest that age itself remains a stronger determinant of brain MDA levels than Spirulina supplementation in this age range. The marked decrease in MDA between S12 and S18 groups (0.843 nmol/mL) is notably larger than the corresponding decrease in control groups (0.416 nmol/mL), which may reflect an interaction between Spirulina's bioactive compounds and the brain's developmental antioxidant trajectory, though this did not reach between-treatment significance.

It is important to acknowledge that the small sample size (n=5 per group) represents a significant limitation of this study. With a total of 30 animals, the statistical power to detect moderate effect sizes is limited, increasing the risk of Type II errors (false negatives). Future studies should employ larger sample sizes (minimum n=8–10 per group), include older age groups (up to 72–96 weeks), extend the treatment duration beyond 29 days, and consider higher doses of Spirulina to fully characterize its neuroprotective potential.

4. Conclusion

Brain malondialdehyde (MDA) levels in young adult male Wistar rats (12–24 weeks) demonstrate a declining trend with age in control groups, with a statistically significant reduction between 12-week and 24-week groups ($p = 0.037$). *Spirulina platensis* ethanol extract at 200 mg/kgBW administered for 29 days does not significantly alter brain MDA levels compared to controls at any age group examined ($p > 0.05$). These findings indicate that the hypothesis of uniform MDA reduction by *Spirulina platensis* in young adult Wistar rats across all age groups is not supported at the dose and duration used in this study. Future investigations should employ higher doses of Spirulina, larger sample sizes, extended treatment durations, and broader age ranges, while also exploring alternative lipid peroxidation markers and mechanistic endpoints to fully characterize the neuroprotective potential of *Spirulina platensis*.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be declared.

Statement of ethical approval

The animal study protocol had obtained Ethical Clearance from Research Ethic Committee of Faculty of Medicine, Universitas Indonesia No. KET- 699/UN2.F1/ETIK/PPM.00.02/2020.

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