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Protective effect of *Diadema setosum* gonad extract against oxidative stress in D-Galactose-induced aging rats

Efek perlindungan ekstrak gonad Diadema setosum terhadap stres oksidatif pada tikus tua yang diinduksi D-Galaktosa

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Abstract

Oxidative stress is a primary mechanism of aging and is characterized by elevated levels of malondialdehyde (MDA). Natural antioxidants are being increasingly explored for the prevention of premature aging. The gonads of the sea urchin *Diadema setosum* contain bioactive compounds, including omega-3 fatty acids, flavonoids, and phenolics, which possess unique ecological adaptations that enhance secondary metabolite production, making them a promising marine antioxidant source. This study aimed to evaluate the effect of *D. setosum* gonad extract on MDA levels in male Wistar rats undergoing d-galactose-induced aging. An experimental post-test control group design was conducted from July to November 2024, involving 24 rats divided into four groups: standard control (K1), positive control (K2), and two treatment groups receiving extracts at doses of 100 mg/kg (P1) and 300 mg/kg (P2). D-galactose was used for aging at 150 mg/kg body weight for eight weeks, followed by oral administration of the extract for an additional eight weeks. MDA levels were measured using ELISA at weeks 0, 6, 10, and 14. The results showed a significant increase in MDA levels in the positive control group (K2) compared to the normal group ($p=0.002$), indicating oxidative stress induction. In contrast, treatment with *D. setosum* gonad extract, particularly in the P2 group, significantly reduced MDA levels ($p<0.05$), approaching those of the normal group. In conclusion, *D. setosum* gonad extract has potential as a natural antioxidant for suppressing oxidative stress associated with aging.

Keywords: Aging, D-galactose model, *Diadema setosum*, marine bioactive compound, oxidative stress

Abstrak

Stres oksidatif merupakan mekanisme utama dalam proses penuaan, ditandai dengan peningkatan kadar malondialdehid (MDA). Antioksidan alami semakin banyak diteliti sebagai upaya pencegahan penuaan dini. Gonad landak laut *Diadema setosum* mengandung berbagai senyawa bioaktif-termasuk asam lemak omega-3, flavonoid, dan fenolik-serta memiliki adaptasi ekologis yang meningkatkan produksi metabolit sekunder, sehingga berpotensi menjadi sumber antioksidan laut yang menjanjikan. Penelitian ini bertujuan mengevaluasi pengaruh ekstrak gonad *D. setosum* terhadap kadar MDA pada tikus Wistar jantan yang mengalami penuaan akibat induksi D-galaktosa. Studi eksperimental dengan desain *post-test control group* ini dilakukan pada bulan Juli hingga November 2024 dengan menggunakan 24 ekor tikus yang dibagi dalam empat kelompok: kontrol normal (K1), kontrol positif (K2), serta dua kelompok perlakuan yang menerima ekstrak dosis 100 mg/kgBB (P1) dan 300 mg/kgBB (P2). Induksi penuaan menggunakan D-galaktosa 150 mg/kgBB selama 8 minggu, dilanjutkan dengan pemberian ekstrak secara oral selama 8 minggu. Kadar MDA diukur menggunakan metode

ELISA pada minggu ke-0, 6, 10, dan 14. Hasil menunjukkan peningkatan kadar MDA yang signifikan pada kelompok kontrol positif (K2) dibandingkan kelompok normal (p=0.002), yang mengindikasikan adanya induksi stres oksidatif. Sebaliknya, pemberian ekstrak gonad *D. setosum,* terutama pada kelompok P2, secara signifikan menurunkan kadar MDA (p<0.05), mendekati kadar kelompok normal. Kesimpulan, ekstrak gonad *D. setosum* memiliki potensi sebagai antioksidan alami dalam menekan stres oksidatif terkait penuaan.

Kata Kunci: *Diadema setosum*, model D-galaktosa, stres oksidatif, penuaan, senyawa bioaktif laut

Introduction

Aging is a gradual biological process characterized by a deterioration in cellular and physiological functions as well as a heightened vulnerability to numerous degenerative diseases, including diabetes, atherosclerosis, and neurodegenerative disorders (Gama et al., 2021; Guo et al., 2022; López-Otín et al., 2023). One of the primary mechanisms involved in this process is the accumulation of oxidative stress arising from the disparity between free radical generation and antioxidant mechanisms in the body (Sobhon et al., 2023). This imbalance can damage essential biomolecules, including lipids, proteins, and DNA, ultimately accelerating tissue damage and organ dysfunction (Guo et al., 2022; Keshavarz et al., 2023; López-Otín et al., 2023; Villareal, 2023).

One commonly used marker for measuring oxidative stress is malondialdehyde (MDA), the end-product of lipid peroxidation (Mas-Bargues et al., 2021). Elevated MDA levels are associated with aging and age-related diseases (Mutlu-Türkoğlu et al., 2003). To experimentally mimic aging conditions, the chronic induction of d-galactose in experimental animals has been widely used (Datrianto et al., 2021). This model leads to increased oxidative stress and systemic inflammation, characterized by elevated MDA levels, which reflects lipid damage caused by free radicals (Azman & Zakaria, 2019; Fatemi et al., 2018; Homolak et al., 2022).

In recent years, there has been growing interest in natural antioxidant sources, including those from marine organisms (Ngoc Nhon Hoang et al., 2023). Among these, the sea urchin, *D. setosum* has emerged as a particularly promising species because of its rich biochemical composition and ecological resilience (Gama et al., 2024; Tulandi et al., 2021). The gonads of this sea urchin contain various bioactive compounds, such as polyunsaturated fatty acids, lipophilic vitamins (A and E), bioactive peptides, carotenoids, flavonoids, and phenolic compounds. These compounds have been reported to possess antioxidant activity, capable of neutralizing free radicals and protecting cells from oxidative damage (Karmilah et al., 2021; Ngoc Nhon Hoang et al., 2023; Tulandi et al., 2021). Members of the phylum *Echinodermata*, including *D. setosum,* are also known to produce *naphthoquinone* pigments, such as spinochromes, which act as free radical scavengers in DPPH assays and play an essential role in lipid peroxidation inhibition and maintenance of cellular redox homeostasis (Ghelani et al., 2022; Moreno-García et al., 2022).

Furthermore, *D. setosum* exhibits unique ecological and biochemical advantages compared to other marine organisms. Its long spines and benthic coral adaptation enable the accumulation of secondary metabolites in response to persistent oxidative stress in its natural reef habitat(Afifa et al., 2018). This ecological specialization enhances its capacity as a distinctive marine antioxidant source, distinguishing it from other marine species that do not experience chronic oxidative stress, such as fish. Therefore, the selection of *D. setosum* as an antioxidant research model is scientifically justified not only by its availability but also by the synergistic interaction of its biological, chemical, and ecological characteristics that support strong and specific antioxidant activity against oxidative stress (Ghelani et al., 2022; Karmilah et al., 2021; Nurkolis, 2025).

This study aimed to evaluate the protective effects of *D. setosum* gonad extract against d-galactose-induced oxidative stress, focusing on MDA levels as the primary indicator. By observing changes in MDA levels at various time points and treatment doses, this study is expected to provide scientific evidence regarding the antioxidant potential of sea urchin gonad extract and its contribution to interventions against the aging process.

In addition to their antioxidant properties, some bioactive compounds in *D. setosum* gonads are known to have anti-inflammatory potential, which can provide dual protection against tissue damage caused by aging (Karmilah et al., 2021). The mechanism of action of these compounds involves activation of the antioxidant signaling pathway Nrf2-ARE and inhibition of the inflammatory pathway NF-κB. Activation of Nrf2 increases the expression of endogenous antioxidant enzymes, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, which work synergistically to reduce free radical levels. In contrast, NF-κB inhibition reduces the production of pro-inflammatory cytokines, such as TNF-α and IL-6, which exacerbates oxidative stress and accelerates cellular aging (Ngoc Nhon Hoang et al., 2023; Ode Salma et al., 2016; Sabilu et al., 2022; Tulandi et al., 2021).

The selection of 100 mg/kg and 300 mg/kg doses of *D. setosum* gonad extract was based on previous studies on marine and echinoderm extracts that demonstrated biological efficacy and safety within this range. Khalil et al. (2023) reported that sea urchin (Diadema savignyi) extract at 50–100 mg/kg provided neuroprotective and antioxidant effects in rats, whereas other marine-derived extracts showed significant antioxidant and anti-inflammatory activities at doses between 100 and 400 mg/kg. Therefore, 100 mg/kg was selected as the effective low dose to represent the minimum biological activity threshold, and 300 mg/kg was chosen as the higher but still safe dose to evaluate potential dose-dependent responses without toxicity. This range allows for the assessment of both efficacy and safety in the D-galactose-induced aging model (Samir et al., 2023; Yim et al., 2017).

Utilizing local marine resources, such as *D. setosum*, as potential bioactive agents to mitigate the aging process represents a strategic approach for developing natural therapies based on local biodiversity. In addition to being an effective and safe solution for anti-aging interventions, this approach supports the sustainable utilization of Indonesia's biological resources. Thus, this study is expected to serve as a foundation for the development of marine-based supplements or phytopharmaceuticals that offer tangible benefits in slowing the aging process in a scientifically and sustainable manner. Although sea urchin gonad extracts exhibit antioxidant activity, their efficacy in reducing oxidative stress in aging models has not yet been fully experimentally validated.

Methods

This study used a laboratory experimental design with an in vivo approach, conducted on male Wistar rats (Rattus norvegicus) aged 6–8 weeks with body weights ranging from 300 to 350 g. All rats were housed under controlled environmental conditions, with room temperature maintained at 22–25°C and a 12-hour light-dark cycle per day. During the maintenance period, the experimental animals were provided with standard feed and drinking water ad libitum (Datrianto et al., 2021). The research protocol was approved by the Health Research Ethics Committee of the Faculty of Medicine, Hasanuddin University (ethics number 580/UN4.6.4.5.31/PP36/2024.

A total of 24 rats were randomly divided into four treatment groups using a computer-generated randomization list to minimize allocation bias, each consisting of six rats, as follows: K1: normal control group (no treatment); K2: negative control group induced with aging using D-galactose alone; P1: treatment group with D-galactose + Diadema *setosum* gonad extract at a dose of 100 mg/kg body weight; and P2: treatment group with D-galactose + *D. setosum* gonad extract at a dose of 300 mg/kg body weight.

Aging was induced by intraperitoneal injection of D-galactose at a dose of 150 mg/kg body weight per day for 6 weeks, followed by 8 weeks of oral administration of *D. setosum* gonad extract via a feeding tube, according to the doses determined for each group (100 mg/kg and 300 mg/kg body weight). These doses were selected based on prior studies on marine-derived antioxidant extracts that demonstrated efficacy and safety within this range, as detailed in the Introduction section. The extract was prepared as a homogeneous suspension in sterile distilled water immediately before administration to ensure stability and dose accuracy.

The *D. setosum* gonad extract used in this study was obtained via maceration using acetone. Detailed phytochemical characterization, including qualitative analysis

of alkaloids, flavonoids, steroids/triterpenoids, phenols, tannins, and saponins, quantification of active antioxidant components (vitamin E, vitamin A, and DPPH radical scavenging activity), and GC-MS profiling were performed.

Blood samples were collected serially at four different time points: week 0 (before treatment or baseline), week 6 (after aging induction), week 10, and week 14 (after extract administration). Blood samples were collected via the retro-orbital vein using a hematocrit capillary tube and centrifuged at 3,000 rpm for 15 min to obtain serum. The resulting serum was stored at −20°C until analysis.

Malondialdehyde (MDA) levels were measured using the Enzyme-Linked Immunosorbent Assay (ELISA) method with a commercial kit specific for rats (rat MDA ELISA kit) following the manufacturer's recommended protocol. The results were measured using a microplate reader at 450 nm. All MDA levels obtained were statistically analyzed using one-way ANOVA, followed by Bonferroni post-hoc tests to evaluate significant differences between groups and time points, with a significance level of $p < 0.05$.

Result and Discussion

Malondialdehyde (MDA) is the end product of lipid peroxidation and is often used as an indicator of oxidative stress levels in biological tissues. Elevated MDA levels indicate cellular damage caused by free radicals, a condition commonly observed during aging and associated with degenerative diseases(Mas-Bargues et al., 2021; Sobhon et al., 2023). In this study, MDA levels were analyzed in two main phases: after aging induction with D-galactose and after administration of *D. setosum* gonad extract to evaluate the efficacy of the extract in reducing the oxidative stress.

Malondialdehyde (MDA) Levels During Aging Induction with D-galactose

To evaluate the success of the D-galactose-induced aging model, MDA levels were measured at week 0 (baseline) and week 6 (postinduction). The purpose of this measurement was to assess the increase in oxidative stress due to the long-term use of D-galactose.

Table 1. MDA levels before induction (week 0)

Groups	MDA Levels (nmol/mL)			
	Min	Max	Mean ± SD	p-value
K1	1.46	2.32	1.95 ± 0.36	0.805
K2	1.43	1.88	1.71 ± 0.19	
P1	0.42	2.14	1.66 ± 0.62	
P2	1.22	2.41	1.71 ± 0.48	

The mean MDA levels of mice at week 0 showed relatively uniform values among groups K1 (1.95±0.36), K2 (1.71±0.19), P1 (1.66±0.62), and P2 (1.71±0.48). The ANOVA test results showed no significant differences in MDA levels between the groups ($F = 0.511$; $p = 0.679$). The results of the Bonferroni post-hoc test also showed that all comparisons between groups were insignificant ($p > 0.05$), confirming that the initial conditions of oxidative stress in all groups were equivalent before the induction and treatment.

Table 2. MDA levels after induction with D-galactose and before intervention with *D. setosum* gonad extract (week 6)

Groups	MDA Levels (nmol/mL)			
	Min	Max	Mean ± SD	p-value
K1	1.31	1.93	1.61 ± 0.20	0.042
K2	1.64	2.36	2.00 ± 0.23	
P1	1.64	2.41	1.95 ± 0.20	
P2	1.62	2.59	2.04 ± 0.31	

MDA levels at week 6 after D-galactose induction showed a significant increase in the treatment (P1 and P2) and positive control (K2) groups compared with the negative control group (K1). The highest mean MDA value was found in group P2 (99.17 ± 7.62 nmol/ml), followed by P1 (97.33 ± 9.39 nmol/ml) and K2 (96.00 ± 13.88 nmol/ml), while K1 showed lower MDA levels (83.00 ± 1.67 nmol/ml). A p-value of 0.026 indicated a statistically significant difference in the results.

These results confirmed the hypothesis that D-galactose-induced aging leads to increased oxidative stress, characterized by elevated MDA levels. This increase indicates lipid peroxidation and cell membrane damage resulting from the action of free radicals. D-galactose is a reducing sugar that forms AGEs and produces ROS, which triggers oxidative stress (Azman & Zakaria, 2019; Datrianto et al., 2021; Homolak et al., 2022).

These findings align with those of Cui et al. (2006), who demonstrated that the administration of D-galactose for six weeks significantly increased MDA levels. Zhang et al. (2014) observed an increase in MDA levels and a decrease in antioxidant capacity following d-galactose administration. Another study by Li et al. (2012) showed that high doses of D-galactose accelerate cell damage through inflammatory and oxidative pathways (Azman & Zakaria, 2019; Datrianto et al., 2021; Homolak et al., 2022; Leyane et al., 2022; Liguori et al., 2018).

The increase in MDA levels in this study is suspected to be due to a disruption in the redox balance caused by excess ROS without compensation from the endogenous antioxidant system. Glycation by D-galactose produces AGEs, which activate RAGE, trigger ROS via NADPH oxidase, and exacerbate lipid peroxidation (Leyane et al., 2022; Liguori et al., 2018; Sharifi-Rad et al., 2020).

Oxidative stress caused by D-galactose is associated with the suppression of the Nrf2 transcription pathway, which regulates the expression of antioxidant enzymes such as SOD, GPx, and catalase. Decreased Nrf2 activation reduces the ability of cells to neutralize ROS (Iakovou & Kourti, 2022). Furthermore, D-galactose causes mitochondrial dysfunction, a significant source of ROS, which exacerbates membrane lipid damage and accelerates cellular aging (Azman & Zakaria, 2019; Datrianto et al., 2021; Sharifi-Rad et al., 2020; Sobhon et al., 2023).

Malondialdehyde (MDA) Levels after Intervention with *Diadema setosum* Gonad Extract

After the induction period, *D. setosum* gonad extract was administered for 8 weeks to evaluate the antioxidant effects of the bioactive compounds it contained. MDA levels were measured again at weeks 10 and 14 to assess the dynamics of oxidative stress reduction after the treatment.

Table 3. MDA levels after 4 weeks of intervention with *D. setosum* gonad extract (week 10)

Groups	MDA Levels (nmol/mL)			
	Min	Max	Mean ± SD	p-value
K1	1.47	1.78	1.63 ± 0.10	0.271
K2	1.44	2.22	1.82 ± 0.33	
P1	1.43	1.96	1.64 ± 0.20	
P2	1.38	2.36	1.87 ± 0.32	

The Shapiro-Wilk normality test indicated that all groups had a normal data distribution, with p-values greater than 0.05. However, Levene's test revealed that the data variances between the groups were not homogeneous (p = 0.021). The results of one-way ANOVA showed the mean MDA levels as follows: K1 (1.63 ± 0.10), K2 (1.82 ± 0.33), P1 (1.64 ± 0.20), and P2 (1.87 ± 0.32). Although MDA levels tended to increase in the K2 and P2 groups, statistical analysis showed no significant difference between the groups (F = 1.405; p = 0.271). The stability of MDA levels in the P1 group, which was close to the K1 value, indicated a potential initial protective effect of low doses against oxidative stress. The Bonferroni post-hoc results also showed that all p-values between groups were not significant (p > 0.05), indicating that the antioxidant effect of the gonad extract may not have been fully apparent at week 10 or that a longer time may be needed for statistical detection of the effect of the extract.

Development was more clearly observed in the 14th week, which was eight weeks after extract administration. Malondialdehyde (MDA) after 8 weeks of intervention with gonad extract (week 14) are shown in Table 4.

Table 4. MDA levels after 8 weeks of intervention with *D.setosum* gonad extract (Week 14)

Groups	MDA Levels (nmol/mL)			
	Min	Max	Mean ± SD	p-value
K1	1.36	1.97	1.64 ± 0.25	0.002
K2	1.72	2.71	2.12 ± 0.33	
P1	1.39	1.78	1.57 ± 0.17	
P2	1.27	1.74	1.56 ± 0.19	

The Shapiro-Wilk test indicated normal data distribution in all groups, and the Levene test revealed homogeneous variance (p = 0.733); therefore, the analysis proceeded with One-Way ANOVA. The test results showed a significant difference in MDA levels between the groups (F = 6.955; p = 0.002). The mean MDA levels in P1 (1.57 ± 0.17) and P2 (1.56 ± 0.19) showed a significant decrease compared to K2 (2.12 ± 0.33), which experienced an increase in oxidative stress. MDA levels in K1 remained

stable (1.64 ± 0.25 g). These findings support the hypothesis that *D. setosum* gonad extract, particularly at doses of 100 and 300 mg/kg body weight, exhibits a significant antioxidant effect by reducing MDA levels associated with aging. This effectiveness also shows a time pattern in which the protective effect becomes more apparent after a sufficiently long period of treatment.

After administration of the *D. setosum* gonad extract, MDA levels showed a downward trend, particularly in the treatment group compared to the control group. Groups P1 and P2 showed a decrease in MDA levels to 92.83 ± 6.70 and 91.17 ± 8.08 nmol/ml, respectively, compared to the positive control group (K2), which increased to 103.00 ± 10.65 nmol/ml. These results indicate the potential antioxidant activity of the *D. setosum* gonad extract in reducing oxidative stress.

The decrease in MDA levels is an indicator of reduced lipid peroxidation, a process that often increases under conditions of oxidative stress. This activity is likely due to the bioactive compounds in *D. setosum* gonads, such as vitamin E, carotenoids, and flavonoids, which act as free radical scavengers. These results are consistent with those of several previous studies, including those by Hu et al. (2010), Liu et al. (2013), and Pangestuti et al. (2018), which showed that compounds from marine biota can reduce MDA levels and improve antioxidant status(Gama et al., 2024; Karnila et al., 2022; Sabilu et al., 2022).

At the molecular level, the antioxidant effects of the *D. setosum* gonad extract are closely linked to the modulation of Nrf2 and NF-kB signaling pathways. Activation of Nrf2 by flavonoids and phenolic compounds enhances the transcription of antioxidant response element (ARE)-regulated genes, including SOD, GPx, and catalase, thereby reinforcing the endogenous defense against ROS. Conversely, suppression of the NF-kB pathway reduces the expression of pro-inflammatory cytokines, such as TNF-α, IL-1β, and IL-6, which are known to exacerbate oxidative stress through ROS generation. This dual regulation, upregulation of Nrf2 and downregulation of NF-κB, restores redox balance and protects cellular components from oxidative damage (Park et al., 2023; Sharifi-Rad et al., 2020; Sobhon et al., 2023; Yim et al., 2017).

The possible molecular mechanisms underlying the antioxidant effects of *D. setosum* gonad extract include the activation of the Nrf2 transcription pathway, which regulates the expression of various antioxidant enzymes, such as SOD, GPx, and catalase. Nrf2 activation by polyphenolic compounds or flavonoids strengthens cellular defenses against oxidative stress and prevents lipid damage caused by ROS. Furthermore, omega-3 content in marine gonads can influence the metabolic pathways involved in inflammation and lipid oxidation, decreasing NADPH oxidase expression and increasing antioxidant expression through epigenetic mechanisms. These processes contribute to the reduced MDA accumulation and improved cellular redox homeostasis (Anggraini Wulandari & Ferdina Warsito, 2022; Karnila et al., 2022; Sabilu et al., 2022).

Additionally, carotenoids and vitamin E present in the gonad extract can stabilize cellular membranes by donating hydrogen atoms to lipid radicals, thereby terminating peroxidation chains. The synergistic interaction of these bioactive molecules may further enhance Nrf2 activation while concurrently inhibiting NF-kB translocation to the nucleus, offering an integrated molecular explanation for the observed decline in MDA levels. This supports the hypothesis that *D. setosum* gonad extract exerts a multitarget antioxidant effect through the concurrent modulation of oxidative and inflammatory signaling pathways (Liguori et al., 2018; Mas-Bargues et al., 2021).

Comparison of Malondialdehyde (MDA) Levels between Groups before and after Intervention with *Diadema setosum* Gonad Extract

Analysis of changes in malondialdehyde (MDA) levels during the aging induction phase showed that the data for all four groups (K1, K2, P1, and P2) were normally distributed, as indicated by the Shapiro-Wilk significance values for all groups ($p > 0.05$), that is, K1 = 0.200, K2 = 0.200, P1 = 0.200, and P2 = 0.200. The homogeneity of variance test using Levene's test yielded a significance value of 0.231, indicating that the variance between the groups was homogeneous ($p > 0.05$).

Table 5. Comparison of MDA levels between groups before and after intervention with *D. setosum* gonad extract

Group	Δ MDA Levels (nmol/mL)			
	Aging Induction with D-galactose		Intervention with *Diadema setosum* Gonad extract	
	Mean ± SD	p-value	Mean ± SD	p-value
K1	0.34 ± 0.25	0.04	0.03 ± 0.25	0.005
K2	0.29 ± 0.36		0.11 ± 0.40	
P1	0.29 ± 0.67		0.38 ± 0.19	
P2	0.32 ± 0.31		0.48 ± 0.32	

A one-way ANOVA of delta MDA values showed a significant difference between the groups ($F = 3.327$; $p = 0.040$), indicating that the aging induction process with d-galactose increased MDA levels in Wistar rats. Group K1 showed a decrease in MDA levels during the aging induction phase of -0.34 ± 0.25, reflecting a normal physiological state without exposure to oxidative stress. In contrast, group P2 showed the highest increase in MDA levels (0.32 ± 0.31), followed by P1 (0.29 ± 0.67) and K2 (0.29 ± 0.36). The increase in MDA levels in the K2, P1, and P2 groups indicated the successful induction of oxidative stress by d-galactose.

The results showed that aging induction using D-galactose successfully increased MDA levels, particularly in groups K2, P1, and P2, compared to the normal control group (K1), which experienced a decrease in MDA levels. MDA is the end product of lipid peroxidation and reflects the level of oxidative stress in the body. The increase in MDA levels in the D-galactose-induced group indicated the accumulation of free radicals due to the artificial aging process. After intervention with *D. setosum* gonad extract, MDA levels decreased significantly, particularly at a dose of 300 mg/kg body weight (P2), indicating potent antioxidant activity of the extract. This decrease reflects improvements in the oxidative status resulting from the bioactive compounds in the extract, including steroids, peptides, and marine phenolic compounds, which are reactive oxygen species (ROS) scavengers (Ngoc Nhon Hoang et al., 2023).

The data showed a significant decrease in MDA levels after the intervention, with a significant ANOVA result ($p = 0.005$), and the Bonferroni test showed a significant difference between P2 and K1/K2. This study aligns with that of Zhang et al. (2016), who demonstrated that compounds from marine animals can reduce MDA levels by enhancing antioxidant enzymatic systems. Jeong et al. (2012) demonstrated an MDA-lowering effect of sea cucumber gamete extract in a mouse model of aging. Kim et al. (2020) found that marine peptides significantly suppressed lipid peroxidation. In contrast to this study, several reports have shown only moderate effects of marine extracts on MDA, possibly because of differences in species and dosages. A unique feature of D. setosum is its gonads, which are rich in marine steroid compounds that have not been extensively studied (El-Sayed Shawer et al., 2022; Ghelani et al., 2022; Karmilah et al., 2021; Park et al., 2023; Tulandi et al., 2021).

The reduction in MDA levels in the intervention group was likely due to the active compounds in the gonad extract, such as echinochrome A, unsaturated fatty acids, and marine phenol derivatives. These compounds play a role in suppress NADPH oxidase activity and promote the activation of endogenous antioxidant systems such as glutathione peroxidase and superoxide dismutase (SOD) (Tulandi et al., 2021). Furthermore, gonad biopeptides are thought to inhibit pro-inflammatory pathways that induce ROS. This combination of mechanisms accelerates the restoration of redox homeostasis in tissues damaged by artificial aging(Gama et al., 2024).

The strength of this study lies in its rigorous quantitative approach and the use of a relevant aging model. Furthermore, the use of local marine biota, such as *D. setosum,* offers valuable bioprospecting opportunities. However, limitations arise from the absence of enzymatic biomarker measurements (such as SOD and GPx) for comparison with MDA and the lack of isolation of active compounds from the extract. The practical implication of these findings is that *D. setosum* can be used as a natural antioxidant supplement. For further development, chronic toxicity studies, long-term in vivo clinical trials, and isolation of bioactive fractions are required to validate the molecular mechanism of action.

Conclusion

This study confirmed the antioxidant potential of *D. setosum* gonad extract in mitigating oxidative stress in D-galactose-induced aging rats, as evidenced by a significant reduction in malondialdehyde (MDA) levels. The most pronounced effect was observed at a dose of 300 mg/kg body weight, supporting the hypothesis that the extract exhibits dose-dependent antioxidant activity.

These findings contribute to the growing scientific evidence regarding the bioactivity of marine-derived compounds and highlight the potential of *D. setosum* gonad extract as a promising natural antioxidant. Future studies are warranted to further elucidate its molecular mechanism, conduct chronic toxicity and pharmacokinetic assessments, and explore its potential application in the development of safe natural health supplements targeting oxidative stress-related aging processes.

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