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RESEARCH ARTICLE

Impact of Dadih Administration on Clinical Features, Histopathology, DNA Oxidative Damage, and Collagen Density in an Aging *Rattus norvegicus* Rat Model

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Abstract

Skin aging is among the most evident manifestations of the overall aging process which is linked to the development of numerous dermatological conditions. One of principal mechanisms underlying aging is oxidative stress. Increasing evidence suggests that dadih contains beneficial probiotic microorganisms, which can help attenuate free radical and enhance endogenous antioxidant capacity. This study was conducted to assess the impact of oral dadih supplementation on clinical appearance, skin histological structure, DNA integrity, and collagen density in a rat model experiencing the aging process. In this experimental study, thirty female Wistar rats were divided into 5 groups randomly, including a normal group, a control group, and 3 intervention groups receiving different treatment regimens. The aging condition was established through intraperitoneal administration of D-galactose at a dose of 150 mg/kg body weight for 8 weeks. Oral administration of dadih commenced 2 weeks prior to the induction phase at doses of 10 g/kg body weight once daily, 7.5 g/kg body weight twice daily, and 10 g/kg body weight twice daily. Evaluation of the anti-aging effects of dadih was conducted using observation of fur condition as a clinical indicator, quantification of oxidative DNA damage through measurement of 8-hydroxy-2'-deoxyguanosine (8-OHdG) concentrations using the ELISA technique, microscopic examination of skin tissue stained with hematoxylin and eosin and determination of collagen density using Sirius Red staining. The findings revealed that administration of dadih improved the external appearance of the animals, characterized by smoother and thicker fur. Histological assessment further demonstrated enhanced epidermal thickness, decreased flattening of rete ridges, and increased compactness of collagen fibers when compared with the untreated control group. Rats receiving dadih exhibited reduced levels of 8-OHdG and significantly greater collagen density. Among the treatment protocols evaluated, the regimen of 7.5 g/kg body weight administered twice daily produced the most pronounced protective effects.

Keywords: Dadih, DNA, Histopathology, Collagen, Aging, ROS, Skin

Introduction

Currently, the number of people aged 60 years or older is increasing. By 2050, it is estimated that the world's population aged over 60 years will double to 2.1 billion [1]. Since 2021, Indonesia has also entered an aging population, with approximately 1 in 10 residents aged 65 or older. In 2022, West Sumatra was one of 8 provinces included in the ageing population

[2]. Aging is a risk factor for most chronic diseases [3,4]. Skin ageing is the most visible manifestation of ageing in the organism. Skin aging not only negatively affect beauty but is also closely associated with the development of various skin diseases [5]. In several studies, 85.5% of the elderly population suffers from at least one dermatological disorder related to skin aging [6]. Clinical manifestations of skin aging accompany ageing in humans, including xerosis cutis, ichthiosis, dermatitis, impaired wound healing, wrinkles, and the appearance of various benign tumors that can affect quality of life [7].

Oxidative stress caused by ROS is a major trigger for aging [8-10]. Excess D-galactose in the body can cause aging by increasing ROS levels, leading to oxidative stress in the body [11]. Increased ROS levels can cause oxidative damage to skin cells, including DNA. The interaction of ROS with DNA strand nucleases produces the oxidative compound/product 8-hydroxy-2'-deoxyguanosine (8-OHdG) [12,13]. 8-OHdG has become a crucial biomarker for assessing oxidative damage to DNA [12,14]. Increased ROS formation in aging skin is a molecular pathway that can lead to increased collagen degradation [15]. This is a risk factor that can contribute to the development of several chronic diseases in old age [4,16].

A growing body of evidence indicates that the gut microbiota contributes to the modulation of oxidative stress in the host. Through the fermentation of dietary fiber in the digestive system, intestinal microbes produce short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate. SCFAs have antioxidant properties and show potential in reducing oxidative stress in the gastric and systemic areas [17]. SCFAs have been associated with reduced oxidative stress and inflammation as they reduce the formation of ROS and pro-inflammatory cytokines [3]. SCFAs not only maintain enterocyte function locally but also exert systemic effect and affects distant tissue organs such as the skin. These effects are linked to the skin through blood circulation, forming the so-called Gut-skin Axis [18]. Due to the many roles of gut microbiota in health, a number of functional foods often use probiotics as active ingredients [19]. Probiotics have been known to have many beneficial effects on health, including their antioxidant activity and ability to prevent and reverse the toxic effects caused by ROS [20]. Probiotics have numerous biological activities related to skin aging, such as antioxidant activity, prevention of collagen fragmentation, skin hydration, maintenance of skin pH balance, and skin barrier function [21,22].

In recent years, research on functional foods containing probiotics as anti-aging agents has been growing [23]. Several studies have demonstrated a link between probiotic-fermented milk or probiotic strains and aging. Research by Li *et al.* [8] showed that milk fermented with *L. plantarum* HFY01 increased the expression of Cu/Zn-SOD, GSH, and GSH-Px genes in the skin of rat with D-galactose-induced aging. Soy milk fermented with *L. plantarum* HFY09 increased the expression of collagen, SOD, CAT, GSH-Px, TIMP1, and TIMP2 in the skin and decreased MMP expression [23]. Dadih is a type of traditional fermented milk native to West Sumatra which utilizes probiotics as a functional ingredient [24]. It is made from buffalo milk fermented naturally in bamboo tubes [25,26]. Dadih contains nutrients and Lactic Acid Bacteria (LAB) as probiotics, which can provide benefits for human health and produce various bioactive components with different physiological effects [27].

Research on the effects of dadih administration on various diseases *in vivo* has been previously investigated, however research on the effects of dadih administration on aging is still limited. Research by Harun et al. [28] proved that dadih has an anti-aging effect on the kidneys. Harun *et al.* [28] concluded that dadih can reduce MDA levels in kidney tissue and reduce renal interstitial fibrosis in aging. Research by Amelia *et al.* [24] found that oral administration of dadih can increase SIRT-1 levels and reduce TNF-α expression, both markers of oxidative stress and inflammation. This study was conducted to assess the effect of dadih in reducing the aging process by evaluating clinical and skin histopathological characteristics, body DNA damage, and skin collagen density in a rat model experiencing aging.

Materials and methods

Materials

This research used the following materials: Female *Rattus norvegicus* (Wistar strain), dadih from Air Dingin, Alahan Panjang, Solok, West Sumatra, D-galactose, supplied by Himedia GRM101-100G, assay

kits for Rat 8-OHdG ELISA (Elabscience) and Picro-Sirius Red (SkyTec, USA).

Identification of Lactic Acid Bacteria (LAB) in dadih

Molecular identification commenced with DNA extraction from lactic acid bacteria, followed by DNA amplification using 16S rRNA primers via PCR method. The amplified products were subsequently visualized through gel electrophoresis.

Dadih dosage

The recommended daily intake of dadih for a 70-kg adult, based on the suggested consumption of fermented milk, ranges from 100 to 200 mL. Given that the density (ρ) of dadih is 1.04 g/mL, this corresponds to an estimated dose of 104 - 208 g per 70 kg of body weight. The conversion factor used to translate the human dose to a 200 g rat is 0.018. Accordingly, the calculated doses for rat are 1.87 g/200 g body weight (0.018×104) and 3.74 g/200 g body weight (0.018×208). Thus, the administered dose of dadih in rats was equivalent to 0.935 g (approximately 1 g) per 100 g of body weight, corresponding to 10 g/kg body weight [29].

Treatment of experimental animals

Female *Rattus norvegicus* rats, aged 2 - 2.5 months and weighing 180 - 200 g, were obtained from the IRATco animal laboratory provider. The rats underwent 7-day acclimatization and were subsequently randomized into 5 groups of 6 rats each (**Table 1**). The control group (N) received neither D-galactose induction nor dadih administration. The positive control group (C+) underwent D-galactose induction without dadih treatment. Three treatment groups, all D-galactose-induced, received dadih at 10 g/kg BW (once daily) in T1, 7.5 g/kg BW (twice daily) in T2, and 10 g/kg BW (twice daily) in T3. Dadih suspension in distilled water (1 mL/100 g BW) was administered via oral gavage from the first day of the study. Starting from the third week (2 weeks after dadih administration), all rats except the normal (N) group underwent ovariectomy followed by intraperitoneal D-galactose induction (150 mg/kg BW) for 8 weeks. Dadih administration was continued until the study was completed. On day 30 of D-galactose induction, a sterile punch biopsy was performed on the rats' skin with a diameter of 1 cm and blood was collected through the retro-orbital plexus. On the last day of the study, day 60 of D-galactose induction, the rats were fasted for 12 h before terminal euthanasia for blood and skin tissue collection. Each group was treated as shown in **Table 1**.

Table 1 Division of model rat groups

Group	Treatment
Normal (N)	Healthy rat without modelling
Positive control (C+)	Rats induced with D-galactose 150 mg/kg BW intraperitoneally without treatment daily
Treatment1 (T1)	Rats induced with D-galactose 150 mg/kg BW intraperitoneally and given dadih at a dose of 10 g/kg BW (once daily)
Treatment2 (T2)	Rats induced with D-galactose 150 mg/kg BW intraperitoneally and given dadih at a dose of 7.5 g/kg BW twice daily
Treatment3 (T3)	Rats induced with D-galactose 150 mg/kg BW intraperitoneally and given dadih at a dose of 10 g/kg BW twice daily

Serum 8-OHdG (8-Hydroxydeoxyguanosine) examination

Blood was drawn by inserting a hematocrit tube into the retroorbital plexus. Serum was separated from whole blood by centrifugation at 4,000 rpm for 15 min at 4 °C. The 8-OHdG levels in rats serum were examined using the 8-OHdG (8-Hydroxydeoxyguanosine) ELISA Kit according to the procedure in the 8-OHdG Kit manual (Elabscience® EL-0028, China). This ELISA kit used the Competitive ELISA principle.

Serum examination was performed on days 30 and 60 after D-galactose administration.

Skin histopathology analysis

The histopathology analysis was performed using H&E staining to assess overall skin texture and Sirius red staining to examine collagen fibers primarily. On the 45th day, the dorsal skin tissue was obtained using a sterile 1 cm punch biopsy, pressed perpendicular to the skin surface until it penetrated all layers of the skin. On the 45th and 75th days, 2×2 cm^2 of dorsal skin tissue was immediately taken and fixed in a tissue fixative solution. Next, the tissue processing stage was carried out by preparing paraffin blocks through dehydration, clearing, and embedding. The dehydration process was carried out using a Thermo ScientificTM machine (type STP 120 Spin Tissue Processing), the clearing process was carried out with Xylene, and the next process was embedding with a Miles Scientific Sakura Tissue-Tek Thermal Console 4585. The paraffin blocks were cut using a Leica RM2235 microtome at 4 - 5 µm, then stained with Hematoxylin-Eosin according to Merck Laboratory Procedures. To assess the collagen density in the preparation, Picrosirius Red staining was performed by ScyTek Laboratories Inc. The preparations were observed at 100× and 400× magnification in 5 different planes using a Zeiss Primostar brightfield microscope and Lumenera Infinity-1 camera. Collagen density, indicated by the red color, was measured using ImageJ (ImageJ v1.49 software, National Institutes of Health, Bethesda, MD, USA). Collagen density was calculated as the percentage of red collagen pixels compared to the total tissue pixel area [30,31].

Research ethics

Ethics clearance was obtained from the Ethics Committee of the Medical Faculty of ANDALAS University, No: 479/UN.16.2/KEP-FK/2025.

Statistical analysis

Statistical analysis was conducted employing the Kruskal-Wallis test using SPSS 27.0 and GraphPad Prism 10 programs. All data are reported as mean ± standard deviation (SD). Statistical significance was determined at a threshold of $p < 0.05$. Group comparisons were subsequently assessed through Dunn's tests.

Results and discussion

Lactic Acid Bacteria (LAB) in dadih

The sequencing results of this study revealed that the Lactic Acid Bacteria (LAB) present in dadih from, Solok Regency, West Sumatra, as determined by 16S rRNA sequencing, is *Lactobacillus plantarum*. Previous research by Harun *et al.* [28] also found that Water Dadih from Alahan Panjang, Solok Regency, West Sumatra, contains *Lactobacillus plantarum*. LAB is a large group of beneficial bacteria with relatively similar properties. LAB isolated from dadih has the potential to be a natural, nonpathogenic probiotic, capable of surviving in a medium with low pH and high bile salt concentrations, and exhibiting antibacterial activity [26,32].

Clinical and histopathological features of rats skin

Clinical features of the fur showed that normal (N) rats had smooth, clean, yellowish-white fur, while the skin of the positive control rats (C+) induced with D-gal showed dry, rough, falling fur, and a brownish-yellow color (**Figure 1)**. The administration of dadih to the treatment groups (T1-T3) resulted in smoother, thicker fur compared to the (C+) group.

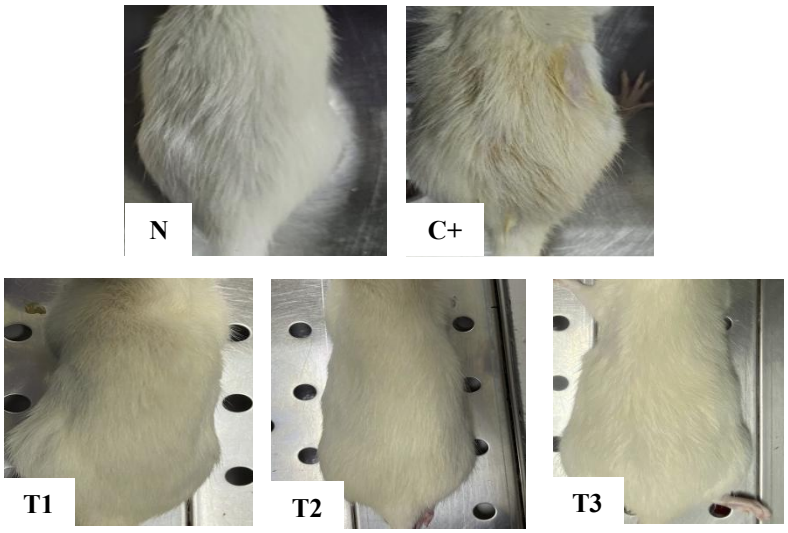


Figure 1 Differences in clinical features of *Rattus norvegicus* rats in the normal (N), positive control (C+) (D-galactose induction), Treatment 1 (T1), Treatment 2 (T2), and Treatment 3 (T3).

The histopathological features of H&E staining in normal rats show a thick epidermis, rete ridges within normal limits with a clear dermo-epidermal junction, and dense collagen in the dermis. In positive control (C+) induced by D-gal, the epidermis is thinned, with some flattening of the rete ridges, thinning of the dermo-epidermal junction, and reduced collagen structure in the dermis (**Figure 2**). Treatment groups (T1 - T3) given orally dadih showed increased epidermal thickness, reduced rete ridge flattening, and denser collagen fibers. Furthermore, rats in treatment group 3 had micrographs that were more similar to those of normal rats.

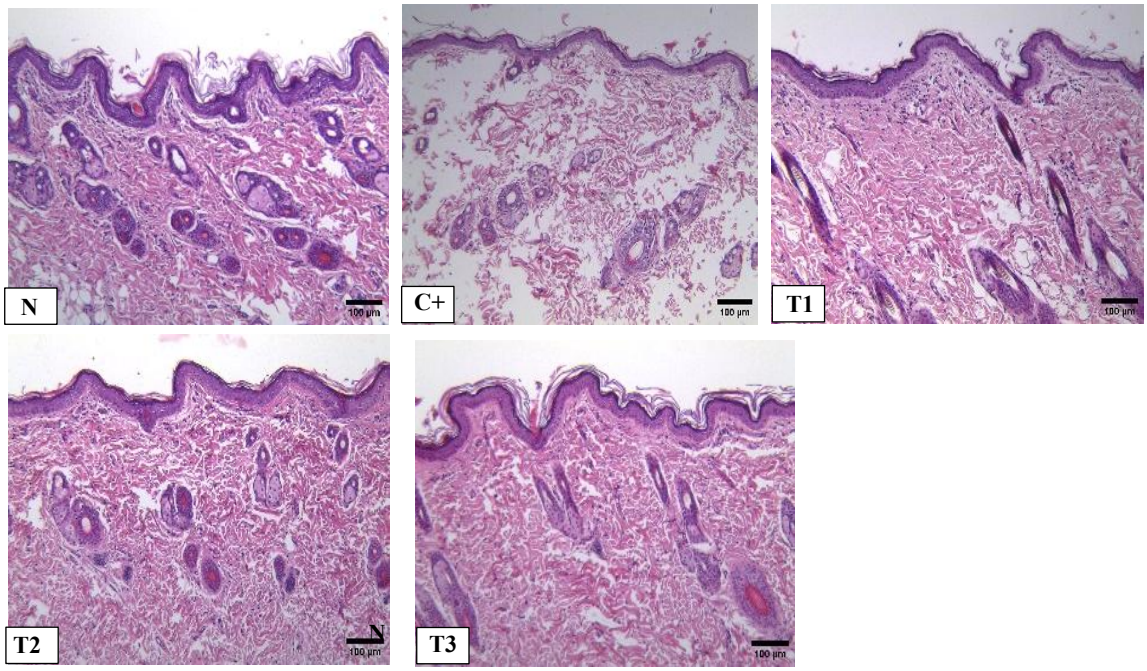


Figure 2 Differences in histopathological features of the skin using Hematoxylin & Eosin staining (100×magnification, 100× scale bar). Normal group (N); no intraperitoneal injection of D-galactose (150 mg/kg BW; Group C+: intraperitoneal induction of D-galactose (150 mg/kg BW); Treatment group 1 (T1); Treatment group 2 (T2); Treatment group 3 (T3).

In this study, an aging rat model was created using D-galactose. D-galactose has low toxicity and a slow onset of action and can stimulate the body to produce large amounts of free radicals [33]. The galactose-induced ageing model is an ideal model for research on anti-aging interventions, because it mimics the characteristics of natural ageing and shares the same morphology and molecular features as those of normal aging rats [34,35]. The clinical and histopathological features of D-galactose-induced mice in this study are consistent with the findings of Rosmarwati *et al.* [36], who reported that intraperitoneal D-galactose injection for 8 weeks resulted in aging changes in young rats that approached those of old rats, clinically and histopathologically. This is similar to the clinical and histopathological findings in naturally aged 18-month-old rats, namely hair loss, yellowish and brownish pigmentation in some areas, and histopathological thinning of the epidermis, flattening of the rete ridges, and reduced collagen [36]. Research by Zhou *et al.* [23] also found that the control group showed a much thinner dermis, fewer collagen fibers, and greater damage than the normal group. Histological changes in the structure of the epidermis of aging skin include decreased epidermal thickness, lower lipid content, and a dermo-epidermal junction are reduced [37]. Histological analysis is often used to assess structural changes in tissues, including skin tissue and to diagnose pathologies such as ageing. Research by Zhao *et al.* [37] showed that a group of mice treated with D-galactose (120 mg/kg/bw) for 6 weeks had decreased collagen fiber content and a blurred boundary between the dermis and epidermis.

Ovariectomy, which is the surgical removal of the ovaries resulting in artificial estrogen deficiency, was also performed in this study to accelerate the aging process in the rat aging model [38,39]. The study by Ivanishchev *et al.* [38] performed ovariectomy on female mice aged 2 months, resulting in significant changes in the epidermis and dermis of the young ovariectomized animals. This procedure accelerated the aging process both in the body in general and in the skin specifically. Ovariectomy also accelerates spontaneous skin aging by decreasing collagen biosynthesis by dermal fibroblasts, significantly reducing type I and type III collagen levels, and accelerating skin atrophy [39-41].

Body weight and organ index in rat

The D-galactose induced model group demonstrated a significantly lower body weight compared with the other groups ($p = 0.041$, $p < 0.05$). Significant variations in brain and spleen indices were observed among the treatment groups ($p = 0.025$ and $p = 0.009$, respectively; $p < 0.05$). In contrast, no statistically significant differences were detected in kidney and heart indices between groups. The brain index in the C+ group was markedly reduced, whereas the spleen index was significantly elevated compared with the normal group (N). Moreover, all treatment groups showed a significant reduction in spleen index relative to the C+ group ($p < 0.05$).

Table 2 Body weight and organ index by different treatments.

Group	Weight (g)	Organ index			
		Heart	Kidney	Spleen	Brain
N	224.71 ± 5.24*	0.31 ± 0.04	0.36 ± 0.05	0.67 ± 0.44*	0.85 ± 0.02*
C+	214.06 ± 4.78	0.35 ± 0.04	0.35 ± 0.03	1.27 ± 0.20	0.79 ± 0.02
T1	222.85 ± 20.14	0.33 ± 0.04	0.38 ± 0.04	0.74 ± 0.23*	0.80 ± 0.06
T2	226.44 ± 10.75*	0.34 ± 0.04	0.36 ± 0.01	0.73 ± 0.17*	0.79 ± 0.10
T3	228.83 ± 11.72*	0.31 ± 0.03	0.36 ± 0.03	0.75 ± 0.14*	0.76 ± 0.04

Note: Data are expressed as mean ± SD (n = 6). Organ index = (organ weight/body weight)×100. Inter-group differences were analyzed using Kruskal-Wallis test followed by Dunn's test; * shows a significant difference at $p < 0.05$ compared to the C+ group. *$p < 0.05$.

Alterations in body weight and the mass of vital organs, such as the brain and spleen, serve as key indicators of the aging process in animals. Aging generally causes weight loss across most organs, which can affect the body's immune system and metabolism [42]. This study aligns with Ge *et al.* [42], who found that the group injected with D-galactose had significantly lower body weight than the other groups, and no significant differences in kidney index were observed among the treatment group. The decrease in brain index aligns with the study by Lin *et al.* [43], which found that the D-galactose-induced group showed a significant decrease compared to the normal group. The nervous system in brain tissue is an important regulatory system in the human body and is also one of the systems most affected by aging. The formation of reactive oxygen species (ROS) in D-galactose induced immune dysregulation and tissue damage, which are factors contributing to splenomegaly [44]. Low-grade chronic inflammation ("inflammaging") triggers an increase in pro-inflammatory cytokines such as IL-6, TNF-α, and iNOS in the spleen, which promotes hyperplasia and the accumulation of immune cells [45].

DNA oxidative damage analysis

The 8-Hydroxy-2'-deoxyguanosine (8-OHdG) is a biomarker of DNA oxidative damage, and measurement of 8-OHdG levels is used to evaluate oxidative stress [12,14]. In this study, there was a significant difference in 8-OHdG levels between treatment groups on both days 30 and 60 ($p < 0.05$) (**Table 3**). This study found that the highest 8-OHdG levels were in the positive control group (C+), but this indicator was completely reversed in the normal group (**Figure 3**). The duration of D-galactose induction also affected 8-OHdG levels in the control group (C+). On day 60, the 8-OHdG levels in the (C+) group increased by 56% compared to day 30**.** Giving dadih can reverse changes in 8-OHdG levels induced by D-galactose administration. The levels of 8-OHdG in groups T1, T2, and T3 on day 30 were 0.95%, 10.00%, and 21.07% lower than those in group (C+), respectively. The levels of 8-OHdG in groups T1, T2, and T3 on day 60 decreased by 59.30%, 61.28%, and 54.42% lower than the results in group (C+) (**Table 3**).

Table 3 8-Hydroxy-2-deoxyguanosine (8-OHdG).

Variables	Group					*p*-value*
	N	C+	T1	T2	T3	
8-OHdG DAY 30	3.69 ± 0.89	8.40 ± 038	8.32 ± 1.17	7.56 ± 1.26	6.63 ± 2.07	0.002
8-OHdG DAY 60	3.04 ± 0.82	13.12 ± 5.36	5.34 ± 1.30	5.08 ± 2.70	5.98 ± 1.19	0.001

Note: Data are presented as mean ± standard deviation (n = 6), analyzed with Kruskal-Wallis.

Giving dadih for 60 days significantly reduced 8-OHdG levels in the serum of rats in groups T1 and T2 compared to the positive control group (C+) ($p < 0.05$) (**Figure 3**). In addition, the duration of dadih administration also affected the decrease in 8-OHdG levels in the treatment group. On day 60, 8-OHdG levels decreased further from day 30, namely 35.8% in T1, 32.8% in T2, and 9.8% in T3.

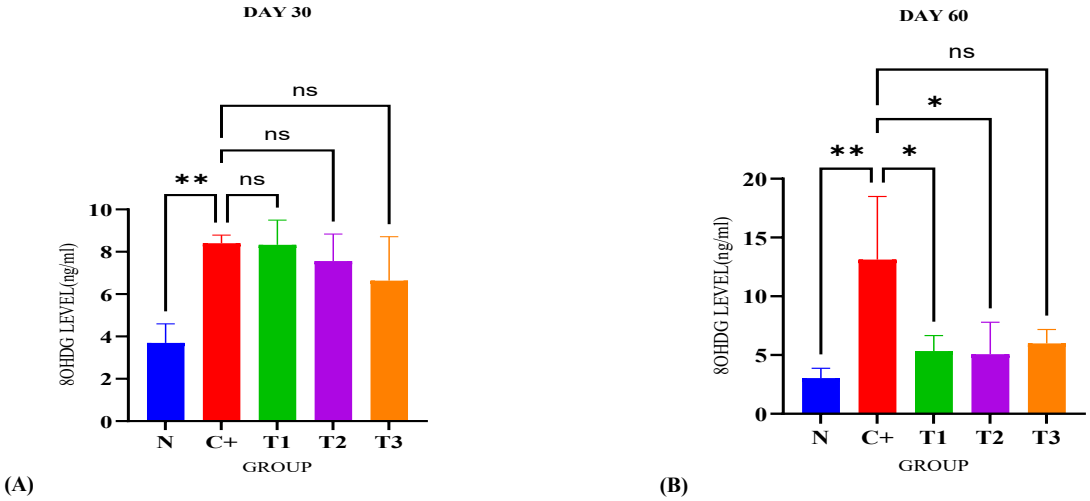


Figure 3 Effect of dadih on changes in 8-OHdG levels in the serum of rats treated with D-gal. 8-OHdG levels in rats serum on day 30 of D-galactosa induction (A), 8-OHdG levels in rat serum on day 60 of D-galaktosa induction (B). Normal group (N): No injection of D-galactose, Positive control group (C+): Injection of D-galactose, Treatment group 1 (T1): injection of D-galactose + dadih 10 g/kg BW once daily, Treatment group 2 (T2): Injection of D-galactose + dadih 7.5 g/kg BW twice daily (T2), Treatment group 3 (T3): Injection of D-galactose + dadih 10 g/kg BW twice daily. Data are presented as mean ± standard deviation (n = 6). Statistical analysis with Dunn's post hoc test. Significant at *$p < 0.05$ compared to C+; ns: not significant ($p > 0.05$).

D-galactose in the body and skin is an important factor that causes oxidative stress through various pathways [46]. Safitri *et al.* [11] showed that the group induced with D-galactose 150 mg/kg BW over 28 days had the worst aging markers, with the lowest levels of the enzyme superoxide dismutase (SOD) and the highest levels of malondialdehyde (MDA), interleukin (IL)-6, and tumor necrosis factor-alpha (TNF-α).

Administration of D-galactose can cause increased accumulation of AGEs. The binding of AGE to the receptor RAGE can increase ROS production by activating NADPH oxidase [47]. Aging is closely related to the reaction of Oxygen Species (ROS) in the body. ROS Radicals can cause damage to DNA, proteins, lipids, and other molecules, which causes a gradual loss of physiological function [48,49]. ROS can directly or indirectly damage cellular DNA and proteins [12,13]. DNA damage can be assessed by measuring the DNA fragmentation index and 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels [50]. The 8-OHdG in biological fluids can be a marker of oxidative stress [49]. This study, found that the levels of 8-OHdG in the C+ group (D-Galactose induction) were higher than the normal group. This is in line with Zhang [51] research, which found that serum 8-OHdG levels increased significantly in D-Galactose-induced model mice compared to normal mice ($p < 0.05$). Li *et al.* [49] research also found that the levels of 8-OHdG in diabetic mice were 3 times higher than in control mice. 8-OHdG levels also increased with age (9 - 26 weeks). 8- OHdG can be a useful biomarker for oxidative stress markers in aging research [49].

The 8-hydroxy-2'-deoxyguanosine (8-OHdG) is one of the main oxidative compounds/products of DNA. 8-OHdG is formed through the interaction of hydroxyl radicals with DNA strand nucleases on guanine bases, where guanine is the base most susceptible to oxidation [12,13]. The 8-OHdG is a biomarker of DNA oxidative damage, and measurement of 8-OHdG levels is used to evaluate oxidative stress. The 8-OHdG assessment can be performed on various biological samples, including serum and urine. Measurement of 8-OHdG levels is highly sensitive and is associated with oxidative stress. 8-OHdG has been studied as a potential marker of age-related damage accumulation in several studies [49].

Measurement of 8-OHdG (8-hydroxy-2'-deoxyguanosine) levels in this study was carried out on serum. The level of 8-OHdG in serum serves as an indicator of cellular oxidative stress that occurs

generally/systemically [12,52]. In the evaluation of anti-aging, biomarkers used must be obtained from various sources to accurately represent systemic and organ changes that occur with aging in an organism [53,54]. Systemic aging affects internal organs; therefore, a comprehensive anti-aging approach is essential to reduce physiological decline [11]. The study by Zou *et al.* [35] stated that to evaluate the anti-aging effects of fermented soy milk with L. fermentum CQPC04 on mice experiencing oxidative aging induced by D-galactose, examinations of organ indices, serum and tissue indicators, and pathological observations were performed.

This study found that dadih containing LAB *L. plantarum* can reduce the levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) in the serum of elderly rats previously induced by D-galactose. This is possible due to the antioxidant activity of dadih obtained from both dadih peptides and LAB *L. plantarum*. Amelia *et al.* [29] stated that dadih possesses probiotic properties, with peptide components that act as antioxidants and can stimulate endogenous antioxidants in the host. Dadih peptides are food components that have biological functions for health. Research has found that several peptides from fermented milk act as antioxidants [55]. Several previous studies also reported that *L. plantarum* can increase the body's endogenous antioxidant activity. This study is supported by Li *et al.* [56], who found that the *L. plantarum* KSFY06 strain isolated from yogurt increased the activity of SOD, GSH-Px, CAT, GSH, and decreased serum and liver MDA in aging mice. Fermented soy milk *L. plantarum* HFY09 can decrease H2O2 reduction, and AGE in skin induced by D-galactose [23]. *L. Plantarum* HFY01 bacteria can increase glutathione, catalase, SOD, GSH-Px activity, and decrease MDA in the liver, brain, and serum of mice with aging induced by D-galactose [8]. Dadih is included in the category of probiotic dairy products because it is a fermented dairy product and contains lactic acid bacteria [26]. Research by Lin *et al.* [48], demostrates that giving probiotics increases SCFA (Butyrate) and decreases 8-hydroxy-20-deoxyguanosine (8-OHdG) in serum. Research by Kang *et al.* [57] found that *L. plantarum* produces SCFA, namely propionic acid (PA), butyric acid. Butyrate and propionate activate the Keap1-Nrf2 signaling pathway [9,58]. Activated Nrf2 activates certain genes to encode enzymes and proteins to protect cells from aging factors [9].

Collagen density

In this study, there was a significant difference in collagen density between treatment groups on both days 30 and 60. The normal group (N) had the highest collagen density compared to the other groups. This indicates that under normal conditions without oxidative stress, collagen structure is maintained, resulting in optimal skin tissue density. In the positive control group (C+), collagen fiber density was lower compared to the normal group (**Table 4**). Dadih administration showed an increase in collagen density compared to the positive control group (C+). The higher the dadih dose and the longer the administration time, the greater the increase in collagen density in the rat's skin (**Table 4**).

Table 4 Collagen density.

Variables	Group					*p*-value*
	N	C+	T1	T2	T3	
Collagen density Day 30	75.86 ± 4.82	52.72 ± 1.53	63.83 ± 6.83	67.49 ± 4.02	71.91 ± 5.04	0.000
Collagen density Day 60	76.38 ± 1.35	65.28 ± 2.31	70.02 ± 1.38	73.53 ± 4.65	75.46 ± 3.44	0.001

Note: Data are presented as mean ± standard deviation (n = 6). Analyzed with Kruskal-Wallis.

The results of this study showed that the collagen density image with sirius red staining on day 30 in the positive control group (C+) was looser/sparser than the normal group (N). In the treatment group given dadih orally with 3 dose variations, the dose group of 10 g/kg BW (once daily) in T1, 7.5 g/kg BW (twice daily) in T2, and 10 g/kg BW (twice daily) in T3. for 30 days, the collagen density image increased compared to the control group (**Figures 4(a)** and **4(b)**). The density percentage in the (C+) group decreased significantly

compared to the normal group (N) on day 30 ($p < 0.001$). The percentage of collagen density in the skin of rats in groups T1, T2, and T3 on day 30 of dadih administration increased significantly, different from the positive control group (C+) (** $p < 0.01$ * * * $p < 0.001$) (**Figure 4(c)**).

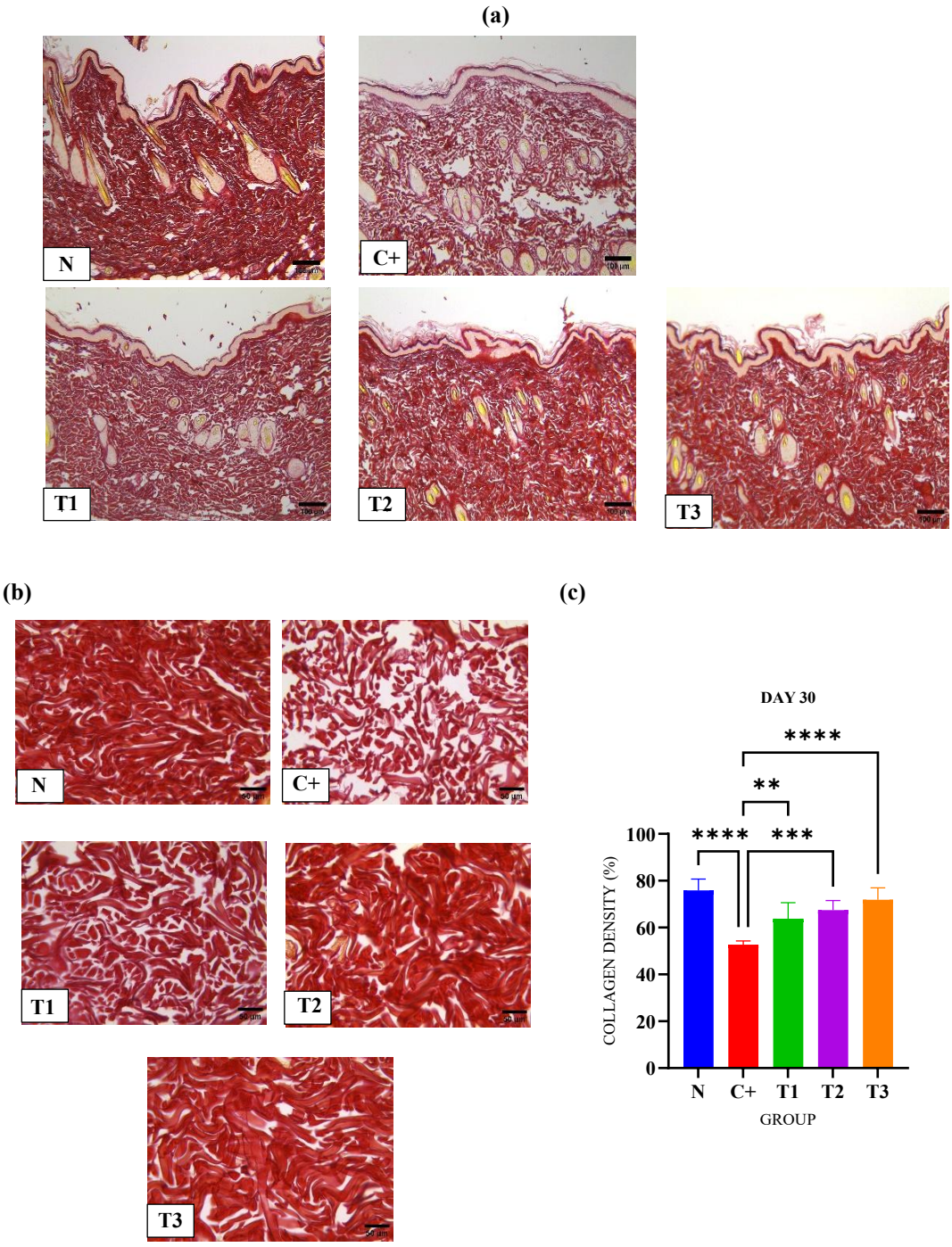
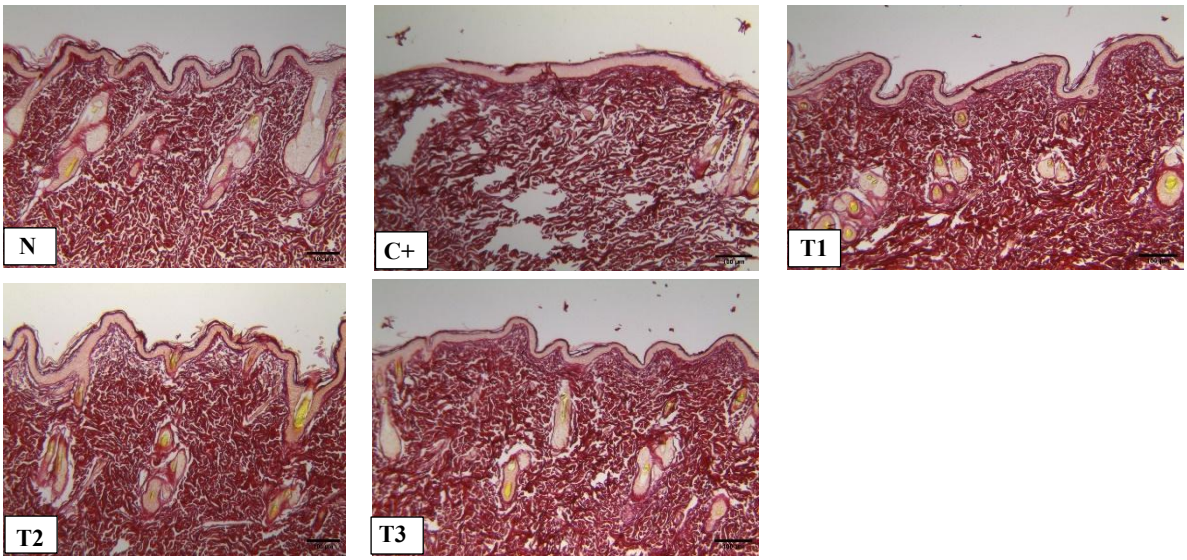


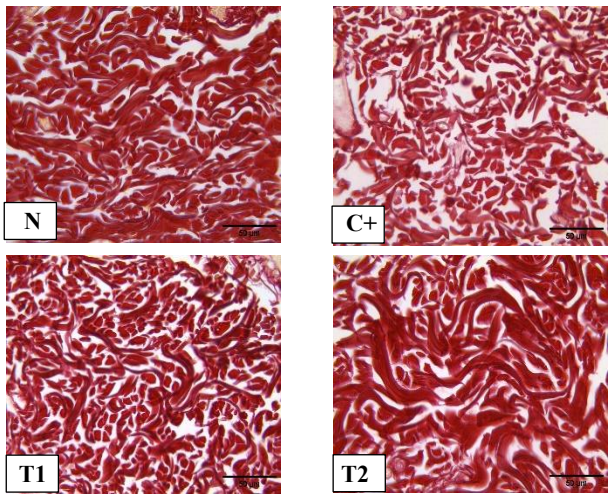
Figure 4 Effect of dadih on changes in collagen density in the skin of rats treated with D-gal for 30 days. Image of skin tissue collagen density with sirius red staining, magnification N 100X, scale bar 100µm (a). Image of skin tissue collagen density with sirius red staining, magnification 400X, scale bar 50 µm (b). Percentage of collagen density in the skin (c). Normal group (N): No injection of D-galactose, Positive control group (C+): Injection of D-galactose, Treatment group 1 (T1): Injection of D-galactose + dadih 10 g/kg BW once daily, Treatment group 2 (T2): Injection of D-galactose + dadih 7.5 g/kg BW twice daily (T2), Treatment group 3 (T3): Injection of D-galactose + dadih 10 g/kg BW twice daily for 30 days. Data are presented as mean ± standard deviation (n = 6). Statistical analysis with Kruskal-Wallis followed by Dunn's post hoc test. Significant at ** $p < 0.01$, * * * $p < 0.001$ compared to C+.

The findings of this study indicated that the histological appearance of collagen density in the positive control group (C+) was more dispersed compared to the normal group (N) on day 60. In contrast, the treatment groups (T1, T2 and T3) receiving oral dadih for 60 days exhibited increased collagen density relative to the C+ group (**Figures 5(a)** and **5(b)**). The percentage of collagen density in the C+ group was significantly reduced compared with the normal group (N) on day 60 ($p < 0.001$). Furthermore, the percentage of collagen density in the skin of rats in groups T2 and T3 after 60 days of dadih administration increased significantly compared with the positive control group (C+) ($p < 0.05$ and $p < 0.01$, respectively) (**Figure 5(c)**). In addition, the duration of dadih administration contributed to a further increase in collagen density in the treatment groups. On day 60, collagen density was higher than on day 30, with increases of 9.7% in T1, 8.9% in T2, and 4.9% in T3. Group T3 demonstrated greater collagen density on both days 30 and 60 compared with the other groups and showed a statistically significant difference relative to the positive control group (C+) ($p < 0.001$, $p < 0.01$) (**Figures 4(c) - 5(c)**).

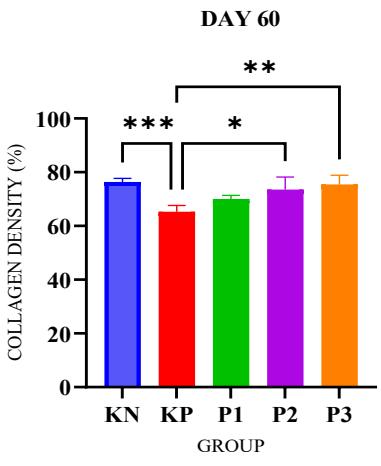
(a)



(b)



(c)



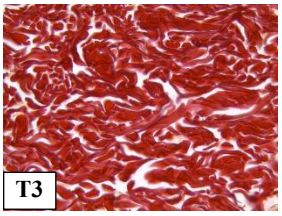


Figure 5 Effect of dadih on changes in collagen density in rat skin induced by D-gal for 60 days. Image of skin tissue collagen density with sirius red staining at 100× magnification, scale bar 100 μm (a); image of skin tissue collagen density with sirius red staining at 400× magnification, scale bar 50 μm (b); percentage of collagen density in the skin (c). Data are presented as mean ± standard deviation (n = 6). Normal group (N): No injection of D-galactose, Positive control group (C+): Injection of D-galactose, Treatment group 1 (T1): Injection of D-galactose + dadih 10 g/kg BW once daily, Treatment group 2 (T2): Injection of D-galactose + dadih 7.5 g/kg BW twice daily (T2), Treatment group 3 (T3): Injection of D-galactose + dadih 10 g/kg BW twice daily for 60 days. Data are presented as mean ± standard deviation (n = 6). Statistical analysis with Kruskal-Wallis followed by Dunn's post hoc test. Significant at $*p < 0.05$, $**p < 0.01$ compared to C+; ns = not significant ($p > 0.05$).

Collagen is the main structural protein in the skin that plays a role in maintaining tissue strength, elasticity, and suppleness. Decreased collagen density generally occurs due to the oxidative stress process in aging, so that the skin becomes more fragile and easily damaged [46]. Sirius red staining was used in this study to observe the image of skin collagen density. This study found that collagen density in positive control (D-Galactose induction) on days 30 and 60 was lower than that of normal rats. This study aligns with the study of Zhao *et al.* [37], which found that a group of mice induced with D-galactose 120 mg/kg/bw for 6 weeks showed low collagen fiber content in the dermis compared to mice from the normal group. This is also in line with the study of Zhou *et al.* [23], which proved that D-galactose induction of 120 mg/kg bw in a rat model intraperitoneally caused a decrease in collagen fiber content compared to the normal group. The normal group had a large number of tightly packed collagen fibers, while the control group had significantly fewer collagen fibers and significant shrinkage.

Skin aging is also influenced by an imbalance between the production of reactive oxygen species (ROS) radicals and the effectiveness of the body's endogenous antioxidant system [59]. Overall, the endogenous antioxidant capacity of the skin decreases with age [60]. In this study, D-galactose administration increased 8-OHdG levels. Increased 8-OHdG is associated with oxidative stress [49]. A study by Zhou *et al.* [23] showed that intraperitoneal D-galactose 120 mg/kg BW induction in a rat model increased oxidative stress, resulting in increased MDA, hydrogen peroxide, and AGEs, and decreased levels of the antioxidants T-SOD, GSH-Px, GSH, CAT, and β-glucan in the rat skin. The accumulation of ROS will trigger a series of molecular chain reactions, including increasing the formation of Activate Activator Protein 1 (AP-1), which will stimulate the transcription process of the Matrix Metalloproteinase (MMP) enzyme, which plays a role in the process of skin collagen degradation [59].

In this study, it was found that giving dadih can increase collagen density in the treatment group compared to the positive control group (C+) starting from the 30th day of giving dadih. On the 60th day of giving dadih, collagen density also continued to increase in all treatment groups, with increases of approximately 9.7% in T1, 8.9% in T2, and 4.9% in T3 compared to day 30. Zhou *et al.* [23] research also demonstrated that the treatment group given milk fermented with *L plantarum* HFY09 had a higher collagen density than the control group. The skin collagen density of mice in the group given milk + *L. plantarum* HFY09 increased significantly, and the final increase approached that of the normal group. The results showed that HFY09 fermented soy milk can effectively reduce skin damage caused by aging caused by D-galactose. Harun *et al.* [28] research using cirrhosis red staining on kidney tissue found that administering dadih to the treatment group reduced the

rate of fibrosis in aging kidneys compared to the control group. Dadih is thought to prevent lipid peroxidation as the components of dadih function as antioxidants, namely peptides and lactic acid bacteria (LAB), which can prevent the formation of ROS [28].

In the current study, D-galactose modeling rats significantly increased 8-OHdG levels and decreased collagen in the skin, indicating that D-galactose induces skin aging. However, in rats induced by D-galactose and given dadih, the 8-OHdG indicator decreased significantly, and the collagen density indicator experienced a final increase approaching the normal group. These findings indicate that dadih containing *L. plantarum* prevents the aging process in rats. *L. plantarum* is one of the LAB that includes probiotics that produce SCFA metabolite compounds that act as antioxidants. Kim's research [61] found that *L. plantarum* reduces skin collagen loss by influencing the activator-1 protein signaling pathway in skin cells and inhibiting MMP-1 expression in dermal fibroblasts. The increase in collagen density in the treatment group given dadih in this study was made possible by the antioxidant properties of LAB *L. plantarum* and peptides, which can reduce ROS in the body, thereby preventing collagen degradation.

This study shows that oral administration of dadih for 60 days significantly prevents the aging process induced by D-galactose, starting from improving the clinical and histopathological characteristics of the skin, reducing DNA damage, and preventing collagen reduction in skin organs. Dadih administration also prevents weight loss and enlargement of the spleen during the aging process. A dose of 7.5 g/kg body weight given twice daily (T2) was found to be effective, as this dose significantly reduced 8-OHdG levels, increased collagen density, increased body weight, and decreased spleen organ index compared to the positive control group (C+). This study only investigated the effects of dadih administration on a limited number of aging indicators and organs. Further studies on the effects of oral dadih administration with D-galactose on other aging indicators and various other organs are needed, as well as further research with clinical trials in humans to comprehensively observe the effects of dadih in inhibiting aging.

Conclusions

This study has demonstrated that oral administration of dadih for 60 days significantly improved the clinical and histopathological characteristics of skin in rats subjected to D-galactose-induced aging. The rats's fur became smoother and thicker, and the thickness of the skin epidermis layer increased, flattening of the rete ridges was reduced, and collagen fibers were denser compared to the control group. The study has also proven effective in reducing 8-OHdG (8-Hydroxydeoxyguanosine) levels, which are an indicator of DNA damage, increasing collagen density in skin, increase body weight and reduce spleen enlargement in rats undergoing the aging process. A regimen of 7.5 g/kg body weight given twice daily produced the most effective protective effect, as it resulted in significant changes compared to the positive control group for all aging indicators in this study. The results of this study offer novel potential for the development of dadih as a functional food that plays a role in preventing the skin aging process. Further studies on oral administration of dadih on other aging indicators and further research with clinical trials in humans are needed to produce a more comprehensive understanding.

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Declaration of generative AI in scientific writing

AI-based tools (Turnitin and Grammarly) were used only minimally for language improvement and similarity checking, without affecting the manuscript's originality, data, or scientific content. All authors have reviewed the study following linguistic corrections and assume full responsibility for the utilization of AI tools.

CRediT author statement

Prima Minerva: Conceptualization, Investigation, Methodology; **Nur Indrawaty Lipoeto**: Data curation, Writing - Original draft preparation; **Jamsari Jamsari**: Visualization; **Tofrizal Tofrizal:** Supervision; **Jamsari Jamsari** and **Tofrizal Tofrizal**:

Software;Validation; **Prima Minerva** and **Nur Indrawaty Lipoeto**: Writing - Reviewing and Editing.

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