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Impacts of D-Galactose on Malondialdehyde, Superoxide Dismutase, and Collagen in Rat Skin

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ABSTRACT

Oxidative stress, primarily mediated by reactive oxygen species (ROS), accelerates skin aging by causing damage, altering antioxidant levels, and promoting inflammation. D-galactose induces oxidative stress by increasing advanced glycation end products and ROS, resulting in cell damage. The present study aimed to assess the effect of intraperitoneal and oral administration of D-galactose on the aging process in rat skin. A total of 27 three-month-old *Rattus norvegicus* were allocated to three groups, each consisting of nine animals. Rats in the control group were not induced with D-galactose (CON). The first treatment group received an intraperitoneal injection of D-galactose at 150 mg/kg body weight (BW; T1), while the second treatment group received oral administration of D-galactose at 500 mg/kg BW (T2). D-galactose was administered for 60 days, and samples of the rat's dorsal skin were collected and examined for malondialdehyde (MDA) levels, superoxide dismutase (SOD) activity, and histological examination using hematoxylin and eosin staining. The clinical features exhibited that the rat's skin treated with D-galactose was dry, rough, and dull, with brownish-yellow fur. Histopathology analysis indicated a significant decrease in collagen density in the treatment group. Intraperitoneal injection of D-galactose significantly increased MDA levels but did not lead to a significant reduction in SOD levels or skin collagen density compared to the control group. The oral administration of D-galactose significantly increased MDA levels but reduced SOD levels and collagen density in skin tissue compared to the control group. The present study indicated no significant differences in SOD, MDA levels, or collagen density between the intraperitoneal D-galactose group and the orally administered group. Oral administration of D-galactose could increase oxidative stress, decrease antioxidant activity, and decrease collagen density in mouse skin. Oral D-galactose can be used as an alternative method to induce skin aging in rats.

Keywords: Aging, D-galactose, Rat, Skin

INTRODUCTION

Skin aging is a multifactorial biological process in which skin undergoes progressive structural and physiological decline (Papaccio et al., 2022). Aging can be caused by different mechanisms, including telomere shortening, oxidative stress, and mitochondrial DNA (mtDNA) mutations. Oxidative stress is a primary mechanism of skin aging through the accumulation of reactive oxygen species (ROS; Yusharyahya, 2021). Different studies have reported that the endogenous antioxidant capacity in the skin decreased with age, and excessive ROS production may disrupt intracellular redox homeostasis, leading to oxidative stress (Yusharyahya, 2021; Papaccio et al., 2022). The accumulation of ROS initiates a series of molecular cascades, including the activation of activator protein 1 (AP-1), which stimulates the production of matrix metalloproteinases (MMPs) and plays a key role in skin collagen breakdown (Ahmad and Damayanti, 2018; Lee et al., 2021). Accumulation of ROS accelerates aging by increasing oxidative damage to DNA and proteins, altering antioxidant enzyme activity, and promoting inflammation in the skin (Mao et al., 2019; Chelliah et al., 2021; Mumtaz et al., 2023). An *in vivo* study on animal models has demonstrated that superoxide dismutase (SOD) and malondialdehyde (MDA) are correlated with the aging process in the body (Mao et al., 2019). Superoxide dismutase is the first endogenous antioxidant to neutralise superoxide radicals and protect cells from oxidative damage. The accumulation of ROS in the system triggers increased MDA levels and decreased SOD, which are biomarkers of oxidative stress in the skin (Mao et al., 2019). Different preclinical studies have explored the effects of medications or interventions on aging. D-galactose-induced aging models, such as rats and guinea pigs, are widely used in such studies because natural aging occurs over a long period (Sriram et al., 2020; Stephania et al., 2024; Gama et al., 2025).

D-galactose is a reducing sugar that converts to galactitol, leading to the formation of advanced glycation end products (AGEs). The AGEs interact with receptors on skin cells, activating the NF-kB signaling pathway and producing ROS (Budni et al., 2016; Sriram et al., 2020; Manus et al., 2023). Excessive administration of D-Galactose can cause

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accelerated biochemical and morphological aging in different organs, including the central nervous system (Stephania et al., 2024). Previous studies on aging in animal models using D-galactose administration have mostly been carried out via the intraperitoneal route (Budni et al., 2016; Sriram et al., 2020). D-galactose has increasingly been used to induce aging in biomedical investigations focused on the molecular and physiological aspects of aging (Guo et al., 2020; Gama et al., 2025). Although intraperitoneal administration of D-galactose effectively mimics certain aging features, this approach has limitations, including stress and discomfort in animals, especially during long-term studies. Furthermore, this method necessitates trained personnel and carries risks of local infection and tissue irritation at the injection site (Martinovic et al., 2025).

The development of aging animal models through oral D-galactose administration remains unexplored (Budni et al., 2016; Sriram et al., 2020). The oral route offers an alternative method for administering D-galactose over an extended period of time (Budni et al., 2016). Furthermore, the rise in blood sugar levels from oral consumption causes the oral model to more accurately mimic real-life aging (Sriram et al., 2020). Studies on methods to delay aging or sustain health during the aging process are advancing rapidly, as these approaches have the potential to enhance the quality of life for older adults (Zhou et al., 2021a). A distinctive feature of the current study is the comparison between intraperitoneal and oral administration of D-galactose, evaluating clinical, biochemical, and histopathological outcomes to determine which route more accurately reflects skin aging. Therefore, the present study aimed to evaluate the effects of intraperitoneal and oral D-galactose on rat skin by comparing clinical indicators, MDA levels, SOD activity, and collagen density.

MATERIAL AND METHODS

Ethical approval

The present study was approved by the Ethics Committee of the Medical Faculty at Andalas University, Indonesia, No. 479/UN.16.2/KEP-FK/2025, on July 18, 2025.

Material

Male *Rattus norvegicus* of the Wistar strain, along with the MDA Kit (Elabsience® Catalogue number E BC-K025-S, China), the SOD Kit (Elabsience® Catalogue number E-BC-K019-S, China), and hematoxylin and eosin (H&E) stain (Merck 1.15938.0025, Germany), were employed in the current study.

Study design

A total of 27 male *Rattus norvegicus*, aged 3 months and weighing 200-300 g, were used in the present study. The animals were housed in individual cages measuring 625.5 cm² by 18.7 cm in height, with five rats in each cage. The cages were equipped with an air-handling unit providing an air velocity of 60 air changes per hour, and temperature and humidity were regulated by an air conditioner, maintaining a temperature of 22 ± 3˚C and a humidity range of 55-68%. The cage bedding consisted of clean, dry, pest-free rice husks sterilized with ultraviolet light. The light source was artificial, following a 12-hour light and 12-hour dark cycle. The experimental animals were fed an 18% protein rodent diet containing 5.7% fat, formulated according to the standard rodent feed formula, and had unlimited access to drinking water. The acclimatization period in the laboratory lasted for seven days. After the acclimation period, the animals were randomly divided into three groups, each with nine rats. The first group was the control group, which was not induced by D-galactose (CON). The second group was the treatment group, which was induced by D-galactose 150 mg/kg BW intraperitoneally (T1; Rosmarwati et al., 2023; Budiningsih et al., 2025), and the third group received oral D-galactose at a dose of 500 mg/kg BW (T2; Sulistyoningrum et al., 2019). The present study was conducted for 60 days.

Sample collection

Rats were anaesthetized using a combination of ketamine (90 mg/kg BW) and xylazine (10 mg/kg BW) administered intraperitoneally. Following the rats' response to painful stimuli, blood samples were collected intracardially. The procedure was carried out until the rats indicated no vital signs and were officially declared dead as a form of terminal euthanasia procedures. The dorsal region was shaved the day before the procedure. A 2 × 2 cm piece of dorsal skin was surgically removed symmetrically from the dorsal area to reduce regional differences. The harvested skin was then split into two equal parts, each maintaining the same thickness. The first part of the skin was immediately placed in Buffered Neutral Formalin (BNF) solution with an adequate tissue-to-fixative ratio for histopathological fixation. In contrast, the second part of the skin was placed in a microtube and frozen for molecular/biochemical analysis. All samples were clearly labeled (animal code, group, storage type) and stored according to the intended analysis.

Skin tissue malondialdehyde examination

Malondialdehyde levels were analyzed using a colorimetric test (TBA Method) according to the instructions in the MDA Kit (Elabsience®E BC-K025-S). The MDA examination measured lipid peroxidation levels in skin tissue samples from experimental rats. The procedure involved collecting the skin tissue, dividing it into small pieces (20 mg), recording the weight, homogenizing it in PBS, and centrifuging at 10,000 g for 10 minutes to isolate the supernatant. Subsequently, the sample was incubated in a water bath at 95-100˚C for 40 minutes, cooled to ambient temperature, and subjected to centrifugation at 3100 g (Thermo Scientific, Germany) for another 10 minutes. The resulting supernatant was then measured at 532 nm using a spectrophotometer (Mindray BA-88A, China).

Skin tissue superoxide dismutase examination

The superoxide dismutase levels in rat skin were examined using the hydroxylamine method according to the instructions in the SOD Kit (Elabsience® E-BC-K019-S, China). The SOD test assessed SOD enzyme activity in tissue homogenates. Homogenize 20 mg of skin tissue in 180 µL of cold PBS at 4˚C, then centrifuge at 10,000 g for 10 minutes, and collect the supernatant. The buffer solution, sample, and reagents were combined in a tube and incubated at 37˚C for approximately 40 minutes. Then, the chromogenic agent was added to the tube and left at room temperature for 10 minutes. The absorbance (OD) of the resulting mixture was measured using a spectrophotometer at 550 nm.

Histopathological analysis of skin

Skin tissue samples measuring 2 × 2 cm were immediately excised and placed in a tissue fixative solution (Buffered Neutral Formalin solution). Subsequently, the tissue processing stage was carried out to prepare a paraffin block, including dehydration, clearing, and embedding. The dehydration process was carried out using a Thermo Scientific™ machine (STP 120 Spin Tissue Processing, Germany) that automatically transferred samples through alcohol solutions of different concentrations, including 70% alcohol (for 1 hour), 80% alcohol (1 hour), 90% alcohol (1 hour), 95% alcohol (1 hour), 100% alcohol I (1 hour), 100% alcohol II (1 hour), and 100% alcohol III (1 hour). Then the clearing process was performed, which involved replacing the dehydration solution with a liquid that was soluble in the embedding medium. The process was carried out with xylene, beginning with xylene I (45 minutes), then xylene II (45 minutes), and finally xylene III (45 minutes). Next, liquid paraffin infiltration was performed on the tissue (paraffin I, paraffin II, and paraffin III) at 60˚C for 30 minutes each. The embedding process involved paraffin-embedding the tissue using the Sakura Tissue TEK III model 4584 tissue embedding machine. The paraffin block was sectioned with a microtome (Leica RM2235, Germany) at a thickness of 4-5 µm, and then stained with H&E. Measurements on H&E preparations were performed with a Zeiss Primostar brightfield microscope (Germany) utilizing a Lumenera Infinity-1 camera from Canada at 100x magnification. Collagen density was assessed by measuring the intensity and density of pink fibers with ImageJ 1.54 g. Collagen density was calculated as the percentage of pixel areas of collagen that were colored pink compared to the pixel area of the entire tissue.

Statistical analysis

Data were presented as mean ± standard deviation (SD). The collected data were tested for normality and homogeneity using the Shapiro-Wilk and Levene's tests. Statistical analysis was conducted using SPSS (version 27.0), employing the Kruskal-Wallis test followed by Dunn's tests. P-values less than 5% were considered statistically significant ($p < 0.05$). Figures were generated by GraphPad Prism 10.

RESULTS

Clinical features

In rats induced with D-galactose intraperitoneally (T1) and orally (T2), the fur appeared brownish-yellow, dry, and rough, and alopecia was observed (Figure 1). The clinical features of alopecia were more prominent in T2 than in T1.

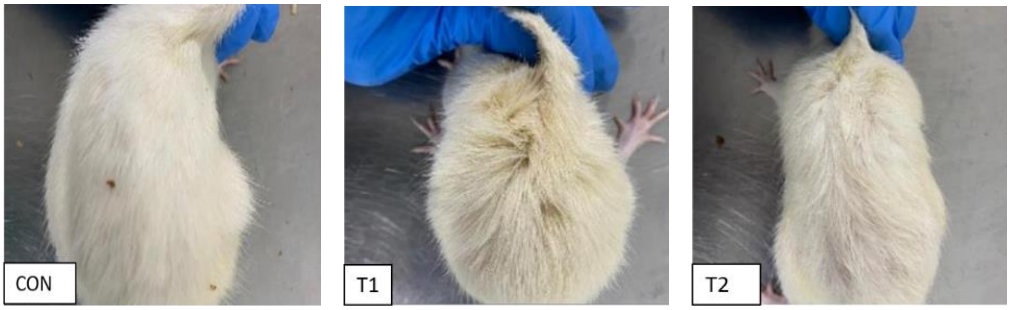

Figure 1. Clinical appearance of 3-month-old male Wistar rats after 60 days of D-galactose administration. CON (Control group): Without induction, T1 (Treatment 1): D-galactose at 150 mg/kg body weight, intraperitoneal, and T2 (Treatment 2): D-galactose at 500 mg/kg body weight, oral.

Malondialdehyde levels

The current results indicated a significant difference in MDA levels among the study groups (p=0.002; Table 1). The present findings demonstrated that MDA levels in T1 (p = 0.04) and T2 (P = 0.002) were significantly higher than in control groups, indicating that both intraperitoneal and oral administration of D-galactose significantly increased MDA levels in the skin of rats. No significant differences were found in MDA levels between T1 and T2 (p = 0.98; Figure 2).

Superoxide dismutase level

The present findings indicated a significant difference in SOD levels among the experimental groups (p = 0.002; Table 1). The current results revealed that T1 did not differ significantly from the control group (p = 0.11). Group T2 differed significantly from the control group, indicating that the oral D-galactose administration significantly reduced SOD activity in the skin of the rat (p = 0.002). The present study indicated no significant difference in SOD levels between T1 and T2 (P = 0.47; Figure 3).

Collagen density and histology in skin tissue

The current results indicated a significant difference in collagen density among the experimental groups (p = 0.039; Table 1). The current results revealed that T1 did not differ significantly from the control group (p = 0.23). However, group T2 did differ significantly from the control group (p = 0.04). The oral D-galactose (T2) administration group significantly reduced collagen density in the rat's skin. The present study indicated no significant difference in collagen density between T1 and T2 (p = 0.10; Figure 4). The present study revealed differences in the microscopic appearance of the skin between the control groups T1 and T2. The control group exhibited a clear dermal-epidermal junction, distinct rete ridges, and dense dermal collagen. In contrast, T1 and T2 indicated age-related changes, including a less distinct dermal-epidermal junction, flattening due to rete loss, and decreased dermal collagen density (Figure 5).

Table 1. Intraperitoneal and oral administration of D-galactose on skin malondialdehyde, superoxide dismutase levels, and collagen density in 3-month-old male rats after 60 days

GROUP	MDA (nmol/mL) Mean ± SD	SOD (u/mL) Mean ± SD	Collagen (%) Mean ± SD
CON	2.42 ± 0.37	42.28 ± 2.49	61.09 ± 0.77
T1	6.07 ± 1.52	33.97 ± 4.28	57.69 ± 0.57
T2	8.00 ± 2.59	29.19 ± 4.83	57.10 ± 4.81

Note: Values are expressed as mean ± SD. P-values (MDA = 0.002; SOD = 0.002; Collagen = 0.039) were derived from the Kruskal-Wallis test comparing all groups. CON: No D-galactose induction (Control), T1: D-galactose at 150 mg/kg body weight administered intraperitoneally, T2: D-galactose at 500 mg/kg body weight administered orally. MDA: Malondialdehyde, SOD: Superoxide dismutase, SD: Standard deviation.

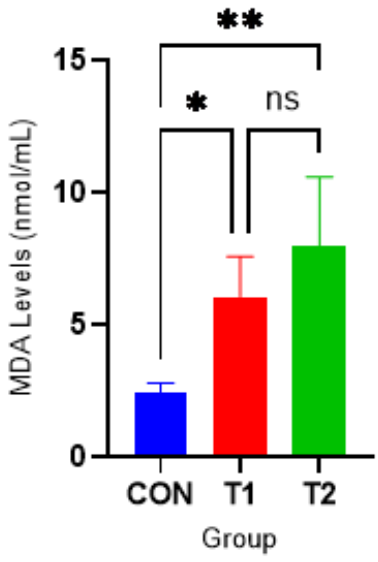

Figure 2. The effect of D-galactose administration on Malondialdehyde levels in 3-month-old male rats after 60 days of treatment. The Malondialdehyde levels in the skin of a control rat (CON); Malondialdehyde levels in rat skin given intraperitoneal D-galactose at 150 mg/kg BW (T1); Malondialdehyde levels in rat skin given D-galactose at 500 mg/kg BW orally (T2). Data are presented as mean ± standard deviation. Statistical analysis with Dunn's post hoc test. NS: Not significant, significant at * $p < 0.05$ compared to the control group.

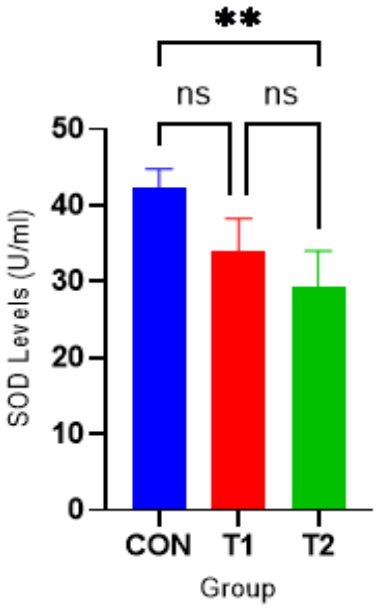

Figure 3. The effect of D-galactose administration on Superoxide dismutase levels in 3-month-old male rats after 60 days of treatment. Superoxide dismutase levels in control rat skin (CON); Superoxide dismutase levels in rat skin injected with 150 mg/kg BW intraperitoneally D-galactose (T1); Superoxide dismutase levels in rat skin given 500 mg/kg BW orally D-galactose (T2). Data are presented as mean ± standard deviation. Statistical analysis with Dunn's post hoc test. NS: Not significant, significant at *p < 0.05 compared to the control group (CON).

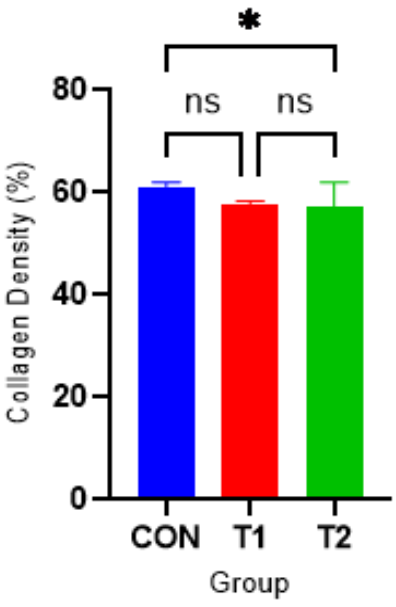

Figure 4. Effect of D-galactose administration on dermal collagen density in 3-month-old male rats after 60 days of treatment. Quantitative analysis of collagen density (%). Data are presented as mean ± standard deviation. Statistical analysis with Dunn's post hoc test. NS: Not significant, significant at *p < 0.05 compared with the control group (CON).

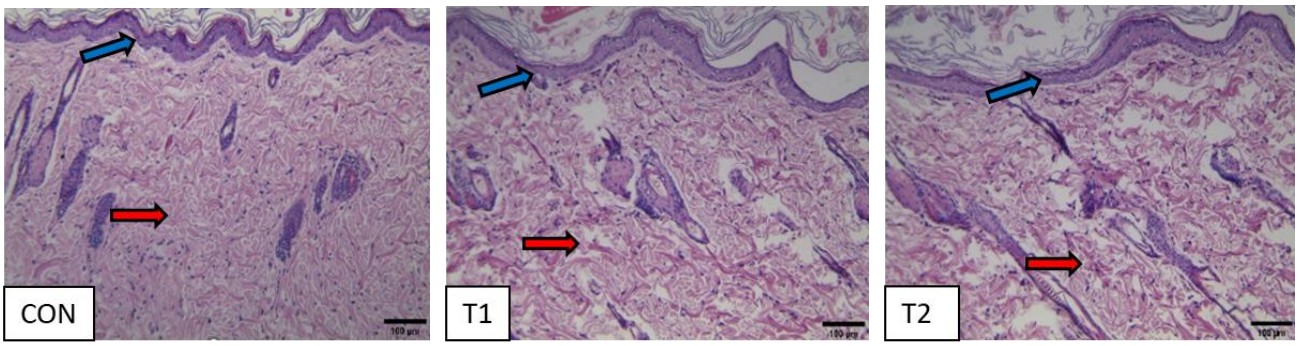

Figure 5. Effect of D-galactose administration on dermal collagen density in 3-month-old male rats after 60 days of treatment. Representative hematoxylin and eosin (H&E)-stained skin sections (100× magnification). CON: No D-galactose induction, showing the rete ridges are clearly visible (blue arrow) and collagen density in the dermis is dense (red arrow). T1: D-galactose at 150 mg/kg body weight administered intraperitoneally, showing flattened rete ridges (blue arrow) and reduced collagen density (red arrow). T2: D-galactose at 500 mg/kg body weight administered orally, showing flattened rete ridges (blue arrow) and collagen density in the dermis is reduced (red arrow).

DISCUSSION

The MDA served as a biomarker of oxidative stress due to lipid peroxidation in cellular membranes. Elevated MDA levels were closely correlated with the processes of aging and the deterioration of skin tissues (Jaffri, 2023; Ibrahim et al., 2025). The current findings indicated that intraperitoneal D-galactose administration elevated MDA levels in skin tissue. The mentioned results aligned with those of Zhao et al. (2019), who observed increased MDA levels and decreased antioxidant activity in the serum, liver, and spleen of rats after 6 weeks of D-galactose induction at 120 mg/kg BW. Additionally, Zhang et al. (2020) reported elevated serum MDA levels in aging rats administered D-galactose at 200 mg/kg BW compared to rats in the control group. Zhou et al. (2021a) demonstrated that intraperitoneal injection of D-galactose at 120 mg/kg BW remarkably increased MDA, hydrogen peroxide, and advanced glycation end-product content in rat skin.

The present study indicated that intraperitoneal injection or oral administration of D-galactose increased MDA levels in the skin, consistent with the findings of Sulistyoningrum et al. (2019), who demonstrated that oral D-galactose at 500 mg/kg BW for 6 weeks increased plasma MDA levels. D-galactose treatment markedly elevated hydrogen peroxide (H_2O_2) levels in rat skin, leading to oxidative stress and enhanced lipid peroxidation, evidenced by higher MDA levels (Mao et al., 2019). D-galactose consumption promotes skin aging through oxidative stress and AGE accumulation (Umbayev et al., 2020; Wang, 2024). D-galactose in the body and skin was an important factor that induces oxidative

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stress through multiple pathways (Umbayev et al., 2020). The binding of AGE to its receptor, the receptor for advanced glycation end-products (RAGE), increases ROS production via NADPH oxidase (Cai et al., 2022). AGE interacts with RAGE on skin cells, thereby activating the NF-kB signaling pathway (Wang et al., 2024). NF-κB activation produces ROS that contribute to cell damage and accelerate skin aging (Cai et al., 2022; Manus et al., 2023). The ROS generated from D-galactose interacted with polyunsaturated fatty acids (PUFAs) in cell membranes, leading to lipid peroxidation and consequently elevating MDA levels (Jaffri, 2023).

The present study demonstrated that intraperitoneal administration of D-galactose did not reduce SOD levels in rat skin. The current findings are consistent with the study conducted by Chen et al. (2016), who reported that a single injection of D-galactose at 500 mg/kg over an 8-week period increased MDA levels, while the antioxidant activities of CAT and GSH-Px in skin remained unaffected (Chen et al., 2016). Petrushev et al. (2015) found that administering D-galactose at 300 mg/kg BW for 42 days to rats of different ages resulted in decreased activities of liver and kidney enzymes. However, decreased activities of liver and kidney enzymes were observed exclusively in mice older than 3 months. The current study differs from that of Li et al. (2021), who demonstrated that intraperitoneal administration of D-galactose at 120 mg/kg BW decreased SOD levels in mouse skin compared with the control group. Additionally, Zhou et al. (2021a) demonstrated that intraperitoneal induction of D-galactose at 120 mg/kg BW decreased T-SOD, GSH-Px, GSH, CAT, and β-glucan levels in the skin of mice (Zhou et al., 2021a). Mumtaz et al. (2023) demonstrated that intraperitoneal injection of D-galactose at 250 mg/kg BW over 60 days resulted in markedly lower activities of antioxidant enzymes, including SOD, CAT, and GSH-Px. Administering D-galactose intraperitoneally alone did not reduce antioxidant levels, as various factors, including the animal's developmental stage, dosage, treatment duration, and rat strain, influenced the outcome (Petrushev et al., 2015; Zhang et al., 2022). Furthermore, the antioxidant defense system demonstrated compensatory plasticity, meaning that when the activity of one antioxidant enzyme decreases, another antioxidant enzyme can increase its activity to maintain sufficient intracellular antioxidant protection (Petrushev et al., 2015).

The present study indicated that oral administration of D-galactose reduced SOD levels in the rat skin. Sriram et al. (2019) reported a decrease in plasma antioxidant glutathione levels following oral administration of D-galactose at 100 mg/kg BW. The SOD enzyme can neutralize ROS, thereby reducing damage to DNA, lipids, and skin proteins associated with aging (Rinnerthaler et al., 2015). Excessive D-galactose in the body is oxidized by galactose oxidase, producing ROS (Li et al., 2019). Excessive ROS or inadequate endogenous defense systems disrupt intracellular redox homeostasis, leading to oxidative stress (Yusharyahya, 2021). Oxidative stress depletes the body's antioxidant enzymes and reduces the system's activity (Li et al., 2019; Shi et al., 2024). Furthermore, elevated levels of D-galactose inhibit the subsequent metabolism of galactitol, resulting in its accumulation within cells. The accumulation of D-galactose can interfere with normal osmotic pressure and impair the antioxidant defense system (Bo-Htay et al., 2018). An *in vivo* study using animal models has demonstrated an association between SOD and MDA and physiological aging (Mao et al., 2019).

Skin aging occurs through different processes, including reduced proliferation of skin cells, decreased synthesis of the extracellular matrix, and increased activity of enzymes that degrade collagen in the dermal layer (Ahmad and Damayanti, 2018). Histological analysis is often used to assess structural changes in skin tissue and to diagnose aging (Huang et al., 2023). The present study indicated that intraperitoneal administration of D-galactose did not markedly reduce collagen levels in rat skin, consistent with the findings of IseMura et al. (2026), who reported that D-galactose induction at 250 mg/kg BW for 6 weeks did not reduce collagen content in rat skin. The present study differs from that of Zhao et al. (2019), who reported that rats induced with D-galactose at 120 mg/kg BW for 6 weeks had low dermal collagen fiber content. Furthermore, Zhou et al. (2021a) reported that intraperitoneal induction of D-galactose at 120 mg/kg BW resulted in fewer collagen fibers than in the control group. Differences in findings regarding the reduction in collagen density after intraperitoneal administration might be attributed to variations in dosage, duration, and rat strain (Cardoso et al., 2015). Furthermore, gender differences may influence outcomes, as may sex hormones such as estradiol, which plays an important role in the oxidative stress response (Isemura et al., 2026). However, only male rats were utilized in the present study.

The present study indicated that oral administration of D-galactose reduces collagen density in rat skin. The current finding is consistent with the results of Sulistyoningrum (2019), who administered 500 mg/kg BW of D-galactose orally and observed a reduction in the number of fibroblasts involved in collagen synthesis in the skin of the treated rats. Skin aging was influenced by the balance between ROS production and the effectiveness of the body's endogenous antioxidant system (Ahmad and Damayanti, 2018). The administration of D-galactose led to excessive ROS production, which can disrupt intracellular redox homeostasis (Yusharyahya, 2021). The accumulation of ROS triggered a series of molecular cascades, thereby increasing AP-1 formation. The AP-1 stimulated transcription of the MMP enzyme, which plays a role in collagen degradation in the skin (Ahmad and Damayanti, 2018). The MMP1 was the primary protease that initiated collagen fiber fragmentation, with collagen types I and III predominating in human skin (Cabral-Pacheco et al.,

2020; Freitas-Rodríguez et al., 2017). Increased MMP production occurred due to the presence of ROS, such as superoxide anions or hydrogen peroxide (Yusharyahya, 2021). The sequence of processes led to increased collagen breakdown and decreased collagen production, thereby forming the pathophysiological foundation of skin aging (Ahmad and Damayanti, 2018). The D-galactose-induced aging model was a suitable approach for anti-aging intervention studies, as this model closely resembled features of natural aging, offered practical advantages in implementation, and was associated with low mortality rates (Azman and Zakaria, 2019; Sulistyoningrum et al., 2019; Hu et al., 2022). The D-galactose-induced aging rat model demonstrated morphological and molecular profiles comparable to those of naturally aging rats (Pantiya et al., 2023). The D-galactose-induced aging model offers advantages, including a short induction period, ease of implementation, excellent reproducibility, and the development of aging signs closely resembling those observed in naturally aged animals (Zhou et al., 2021b; Rosmarwati et al., 2023).

CONCLUSION

Oral and intraperitoneal administration of D-galactose at a dose of 500 mg/kg body weight for 60 days in the present study resulted in a significant increase in MDA and a decrease in SOD and collagen density compared to normal rats. Oral administration of D-galactose could be an alternative method to induce skin aging. The current study administered D-galactose exclusively to male rats and examined the effects of this treatment on aging indicators, which remained limited. Further studies on the oral administration of D-galactose on other aging indicators and female rats should be conducted to produce a more comprehensive understanding.

DECLARATIONS

Acknowledgments

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Authors' contributions

Prima Minerva and Nur Indrawaty Lipoeto were responsible for conceptualizing this article. Jamsari performed the study design and statistical analysis. Prima Minerva collected the data and prepared the manuscript, while Nur Indrawaty Lipoeto reviewed it. Tofrizal performed HE staining and analysis using ImageJ software. All authors approved the final edition of the manuscript for publication.

Ethical considerations

The present manuscript represents an original manuscript by the authors. Ethical issues, including plagiarism, consent to publish, misconduct, duplicate publication, data fabrication, and redundancy, have been reviewed and addressed. 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. Additionally, all authors have confirmed the final edition of the submitted article.

Availability of data and materials

Data supporting the findings of the present study are available from the corresponding author upon reasonable request.

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Competing interests

The authors have not declared any conflict of interest.

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