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Original Article

Effects of porang glucomannan combined with a high-protein diet on oxidative stress, inflammation, and aging markers in D-galactose-induced rats

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Abstract

Aging is a predominant risk factor for several diseases associated with reduced life expectancy. To address this risk factor, several studies have proposed the combined use of porang glucomannan and a high-protein diet to improve various aging markers. The aim of this study was to determine the effects of porang glucomannan and high-protein combination diet as an anti-aging agent. An experimental study using a post-test-only control group design was conducted using Sprague Dawley white rats. The animals were randomly divided into four groups with different treatments: normal control, D-galactose, high-protein diet, and a combination of porang glucomannan and high-protein combination diet. Blood samples were then collected from the ophthalmic vein on day 58 for biomarker measurement using the enzyme-linked immunosorbent assay (ELISA) method. The parameters measured were superoxide dismutase (SOD), malondialdehyde (MDA), interleukin (IL)-6, tumor necrosis factor-alpha (TNF-α), insulin growth factor-1 (IGF-1), NOD-like receptor family pyrin domain-containing protein 3 (NLRP3), growth differentiation factor-11 (GDF11), and α-Klotho levels. The results showed that the combination of porang glucomannan and high-protein diet could improve oxidative stress, inflammation, and aging markers. The analysis of variance (ANOVA) test followed by post-hoc showed significant differences between the combination diet and high protein group (p<0.001). In addition, the average levels of oxidative stress markers (SOD and MDA) in porang glucomannan and high-protein combination group were improved significantly. Similar results were also obtained for inflammatory markers (IL-6 and TNF-α) and aging markers (NLRP3, IGF-1, GDF-11, and α-Klotho). The mean NRLP-3 levels in glucomannan and high-protein combination group were not significantly different compared to control. The study highlights that the combination of porang glucomannan and a high-protein diet effectively improved various aging markers.

Keywords: Aging, porang glucomannan, high-protein diet, inflammation, oxidative stress

Introduction

The elderly, defined as individuals aged ≥65 years, represent a growing global population segment [1]. According to the World Health Organization (WHO), the quality of life during aging is intricately linked to maintaining functional abilities essential for well-being in later life [2]. Life expectancy has increased significantly worldwide, with individuals aged ≥60 years constituting

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12.3% of the population in 2015 and projected to reach 22% by 2059 [3]. In Indonesia, the World Bank reported that 6.68% of the population was aged ≥65 years in 2022 [4]. This demographic shift necessitates a robust health framework to mitigate the prevalence of degenerative diseases, which currently account for 70% of the 57 million annual global deaths [5]. Aging, a natural physiological process marked by structural and functional decline, is accompanied by immune system deterioration, rendering the elderly more vulnerable to diseases [6,7]. Consequently, improving elderly health is imperative to enhance their quality of life globally.

Aging is underpinned by progressive molecular damage driven by oxidative reactions stemming from environmental and metabolic factors, biochemical errors, and nutritional imbalances [8]. Elevated oxidative stress and inflammation exacerbate aging and chronic disease development [9]. Mitochondrial dysfunction, characterized by reduced adenosine triphosphate (ATP) production and increased free radical generation, contributes to this process by elevating oxidative stress markers such as superoxide dismutase (SOD) and malondialdehyde (MDA) [10]. Additionally, immune dysregulation caused by oxidative stress promotes inflammation through mediators like tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-6, while microbiota dysbiosis exacerbates inflammation and reactive oxygen species (ROS) production [11,12]. Gut microbiota diversity and composition influence skeletal muscle metabolism, linking dysbiosis to conditions like sarcopenia and cachexia.

Current anti-aging strategies predominantly focus on aesthetics, neglecting systemic aging that silently affects internal organs. Comprehensive anti-aging approaches are essential to mitigate physiological decline [13]. Emerging research highlights the synergistic potential of glucomannan and high-protein diets in counteracting aging processes [14]. Key biomarkers, including insulin growth factor-1 (IGF-1) [15], NOD-like receptor family pyrin domain-containing protein 3 (NLRP3) [16], growth differentiation factor-11 (GDF11) [17], and α-Klotho [18], have been identified for evaluating these interventions.

Porang (*Amorphophallus muelleri* Blume) has not been widely cultivated because it is an endemic plant that thrives in Indonesian forests. Compared to other tuber varieties, porang contains the highest level of glucomannan compounds and the capacity to generate carbohydrates [19]. Porang glucomannan, a water-soluble non-starch polysaccharide with prebiotic properties, improves gut dysbiosis, oxidative stress, and inflammation associated with aging [20]. Studies in metabolic syndrome models demonstrated porang glucomannan efficacy in reducing body weight and intraperitoneal fat when administered with a high-sugar, high-fat diet [21,22]. Its anti-inflammatory activity, mediated by nitric oxide inhibition in macrophages, underscores its therapeutic potential [23]. Meanwhile, high-protein diets counter oxidative stress through robust antioxidant mechanisms, supporting lipid and glucose homeostasis [24,25].

Combining porang glucomannan and high-protein diets has shown promise in modulating colonic microbiota and improving physiological homeostasis, thus offering a novel anti-aging intervention [25]. Therefore, the aim of this study was to evaluate the efficacy of porang glucomannan and high-protein diets as synergistic anti-aging agents, focusing on their roles in modulating metabolic and inflammatory pathways critical to aging.

Methods

Study design and setting

An experimental study with a post-test-only control group design was conducted to evaluate the effects of a combination of porang glucomannan and a high-protein diet on oxidative stress, inflammation, and aging markers in rats induced with D-galactose. The study was conducted at the Center for Food and Nutrition Studies, Universitas Gadjah Mada, Yogyakarta, Indonesia. All rats were randomly assigned into four groups: the normal healthy control group (control), the D-galactose-induced group without treatment (D-Gal), the D-galactose-induced group receiving a high-protein diet (HP), and the D-galactose-induced group receiving a combination of porang glucomannan (100 mg/200 g body weight) and a high-protein diet (PG+HP). The study began with D-galactose induction for four weeks (28 days), followed by the administration of treatments according to respective groups for another four weeks (28 days). Blood samples were collected from the ophthalmic vein on day 58 to assess the parameters using the enzyme-linked

immunosorbent assay (ELISA) method. The measured parameters included levels of SOD, MDA, IL-6, TNF-α, IGF-1, NLRP3, GDF11, and α-Klotho. The experimental design is illustrated in **Figure 1**.

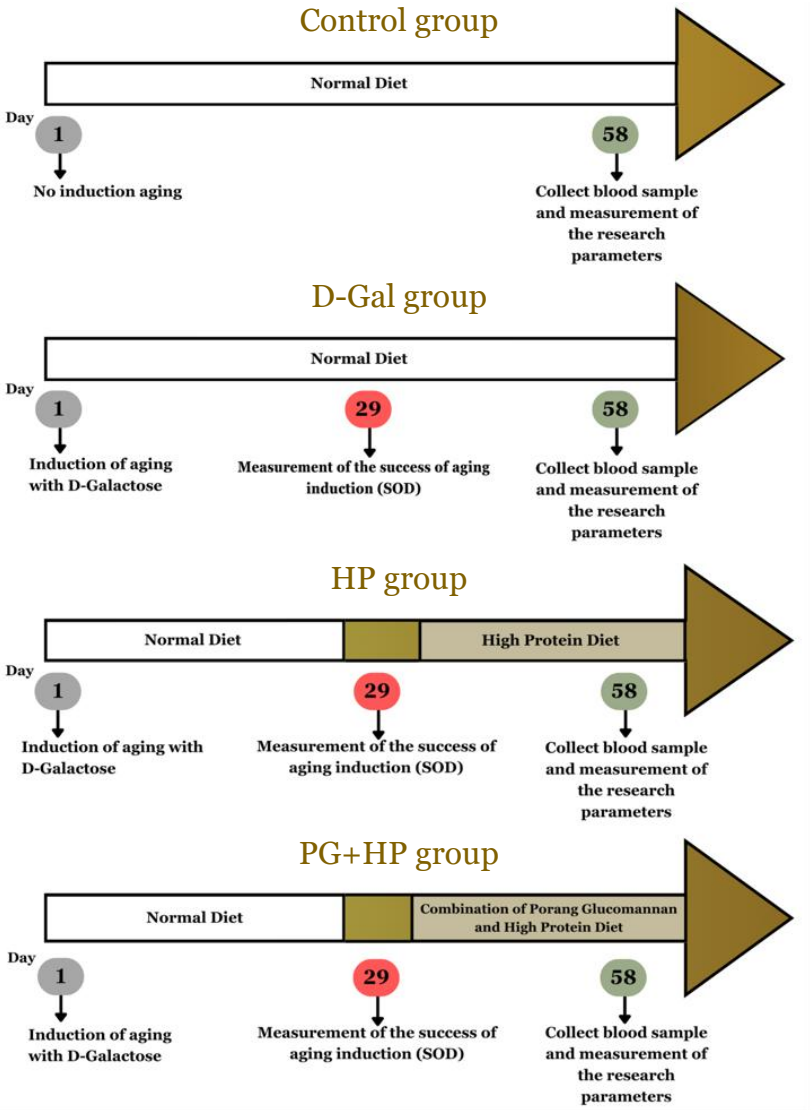

Figure 1. Experimental design of the study. Control group received a normal diet from day one to 58. D-galactose was administered from day 1 to 28 for D-Gal, PG, and PG+HP groups. On day 29, pre-treatment data were collected. From day 30 to 58, D-Gal group only received a normal diet without any treatment. HP and PG+HP group**s** received treatments, high-protein and high-protein with porang glucomannan combination, respectively, from day 30 to 57. Post-treatment data were collected on day 58. Control: healthy control group; D-Gal: induced with D-galactose only; HP: induced with D-galactose and treated with high protein; PG+HP: induced with D-galactose and treated with combination of porang glucomannan and high protein.

Animal and criteria

In the present study, male white rats of the Sprague Dawley strain were used. The rats were obtained from the Center for Food and Nutrition Studies, Universitas Gadjah Mada, Yogyakarta, Indonesia. The inclusion criteria for this study were: (a) healthy male rats with active movement; (b) aged 12 weeks (3 months); (c) body weight of 200±25 grams; and (d) with no anatomical abnormalities. Exclusion criteria included rats that did not exhibit premature aging criteria with low SOD levels after D-galactose induction, died or became ill during the study period.

Sample size and randomization

The sample size for each group was calculated using the WHO formula [26], which prescribes a minimum of five subjects per group with an additional subject included to account for potential losses to follow-up. Consequently, each group consisted of six rats, resulting in a total of 24 study

samples across all groups. Allocation of the samples to experimental groups was performed using a completely randomized design method, ensuring unbiased distribution. Randomization was implemented through a simple draw process to prevent allocation bias and enhance the validity of the experimental outcomes.

Animal acclimatization and housing

All rats were acclimated for seven days to standardize their lifestyle and prevent stress before the study. The rats were housed in individual cages within air-conditioned rooms maintained at a temperature of 18–25°C, 50–70% humidity, with 12-hour light-dark cycle. Water and standard feed were provided *ad libitum* throughout the duration of the acclimatization. During this period, there were no cases of animal dropout, and all animals remained healthy and suitable for the study.

Formulation of porang glucomannan and high-protein diet

The glucomannan utilized in this study was derived from porang (*A. muelleri*) sourced from porang cultivation in East Java, Indonesia. The glucomannan content was quantitatively assessed using gravimetry method at the Testing Laboratory of the Center for Post-Harvest Agricultural Study and Development, Bogor, West Java, Indonesia [22]. The dose used was 100 mg/200 g body weight (BW) based on earlier research that demonstrated efficacy in animals with metabolic syndrome [22]. For the high-protein diet, a 30% protein supplementation was included in the standard diet. The formulation consisted of cornstarch, maltodextrin, casein, L-cystine, sucrose, fats, a comprehensive vitamin and mineral mix, and choline butyrate to ensure balanced nutrient delivery and support metabolic needs.

Study intervention

The initial randomization phase involved assigning the test rats into two distinct groups: 18 rats were subjected to age-conditioning through D-galactose induction at a dose of 150 mg/kg BW over 28 days (day one to 28), while six rats were kept as normal diet as controls (control group). On day 29, the effectiveness of the aging induction was validated by measuring SOD levels in the D-galactose-induced rats.

The rats exhibiting the aged phenotype were then subjected to a second round of randomization, resulting in three experimental groups: the D-Gal group, which consisted of aged rats without treatment; the HP group, which consisted of aged phenotype rats and provided with a high-protein diet; and the PG+HP group, included aged phenotype rats and administered a combination of 100 mg/200 g BW of porang glucomannan along with a high-protein diet. The intervention was sustained for a duration of four weeks, after which further assessments were conducted. After the treatment, all rats were euthanized using cervical dislocation.

Endpoints

The endpoints of this study encompassed various aging-related markers, including markers of inflammation, oxidative stress, and general aging. These biomarkers were quantitatively assessed through blood serum samples obtained from rats on day 58 via the ophthalmic vein. Oxidative stress was evaluated by quantifying SOD activity and MDA levels. Inflammatory markers were evaluated by measuring the IL-6 and TNF-α. Aging-related markers, IGF-1, NLRP3 inflammasome component, GDF11, and α-Klotho were also measured to explore their association with age-related physiological changes. The measurement of the parameters was performed using ELISA techniques, with commercially available ELISA kits specific to each biomarker. The procedure for measuring each marker level was conducted based on the manufacturer's instructions: SOD (Cat. No. K335-100 BioVision, Kampenhout, Belgium); MDA (Cat. No. ER1878, FineTest, Wuhan, China); IL-6 (Cat. No. ER0042, FineTest, Wuhan, China); TNF-α (Cat. No. ER1393, FineTest, Wuhan, China); NLRP3 (Cat. No. RE3548R, Reed Biotech LTD, Wuhan, China); GDF-11 (Cat. No. E0802Mo, BT Lab Bio Assay Technology Laboratory, Shanghai, China); IGF-1 (Cat No. ER0030, FineTest, Wuhan, China); and α-Klotho (Cat No. ER0658, FineTest, Wuhan, China). The results were read at a wavelength of 450 nm using an ELISA reader. The measurement results of each were expressed as follows: SOD (%), MDA (nmol/mL), IL-6 (pg/mL), TNF-α (pg/mL), IGF-1 (pg/mL), NLRP3 (ng/L), GDF11 (ng/mL), and α-Klotho (pg/mL).

Statistical analysis

The data on SOD, MDA, IL-6, TNF-α, IGF-1, NLRP3, GDF11, and α-Klotho levels were analyzed and visualized using GraphPad Prism 9 software (GraphPad Software, Boston, MA, USA). Statistical significance was set at α=0.05 for hypothesis testing. Prior to analysis, the normality of the data was assessed using the Shapiro-Wilk test, and homogeneity of variance was evaluated using Levene's test, yielding *p*-values ≥0.05, indicating normal distribution and homogeneous variances. Statistical analysis was performed by analysis of variance (ANOVA) test followed by a Post Hoc test. Data were analyzed and visualized using GraphPad Prism 9.

Results

Effect of D-galactose induction on SOD levels in aging experimental animals

The SOD levels following D-galactose induction were measured among the groups to assess the presence of an aged phenotype induced by D-galactose. SOD levels were significantly higher in the control group (82.84±3.18%) compared to the D-Gal (27.21±2.43%), HP (28.68±2.09%), and PG+HP (27.99±1.88%) groups (**Figure 2**). Based on these results, D-galactose induction was successful, as all three induced groups showed a decrease in SOD levels (**Figure 2**). Statistical analysis showed no significant differences in SOD levels among the three D-galactose-induced groups, indicating the premature aging model occurred successfully at a similar level in all these groups.

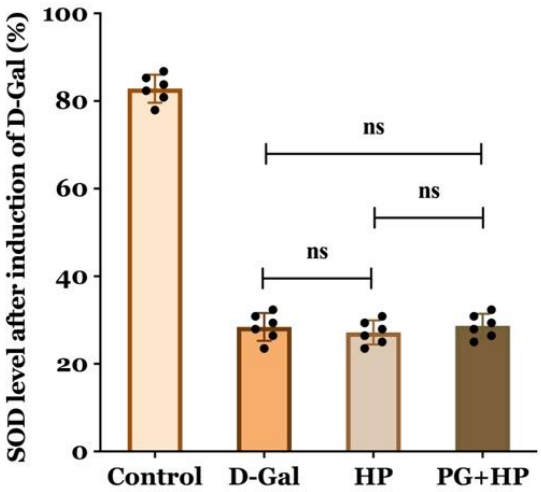

Figure 2. Level of superoxide dismutase (SOD) levels following D-galactose-induced aging. Control: healthy control group; D-Gal: induced with D-galactose only; HP: induced with D-galactose and treated with high protein; PG+HP: induced with D-galactose and treated with combination of porang glucomannan and high protein. Ns: non-significant.

Porang glucomannan combined with a high-protein diet improved oxidative stress, inflammation, and aging markers in D-galactose-induced rats

The effects of porang glucomannan combined with a high-protein diet on markers of oxidative stress, inflammation, and aging were examined. D-galactose administration significantly decreased SOD levels in rats, as measured on day 29 (**Figure 2**). In the D-Gal group, which received only a normal diet until day 57, there was no improvement in SOD parameters, as evidenced by persistently low SOD levels after treatment (**Table 1**). In contrast, the HP group and the PG + HP group showed improvements.

The results indicated that the control group consistently exhibited the best marker levels across all parameters compared to other groups (**Table 1**), whereas the D-Gal group had the worst outcomes. Notably, the PG+HP group achieved superior results on average for all markers compared to the HP group (**Table 1**). Statistical analysis using one-way ANOVA showed that there were significant differences ($p<0.001$) in the mean levels of SOD, MDA, IL-6, TNF-α, NLRP3, IGF-1, GDF-11, and α-Klotho among the groups. These findings confirm that porang glucomannan combined with a high-protein diet positively influenced oxidative stress, inflammation, and aging markers.

Table 1. Comparison of oxidative stress, inflammation, and aging markers among groups

Variables	Groups, mean±SD				*p*-value [a]
	Control	D-Gal	HP	PG+HP	
SOD (%)	84.79±2.71	24.88±3.36	71.01±3.78	77.29±3.13	<0.001
MDA (nmol/mL)	2.26±0.16	9.32±0.42	3.82±0.38	2.98±0.14	<0.001
IL-6 (pg/mL)	42.29±0.88	71.68±0.72	50.58±0.71	45.14±0.46	<0.001
TNF-α (pg/mL)	5.87±0.10	15.94±0.43	7.84±0.11	6.49±0.38	<0.001
IGF-1 (pg/mL)	24.56±0.56	41.10±0.69	31.35±0.52	25.86±0.55	<0.001
GDF-11 (ng/mL)	129.0±11.95	29.02±2.42	75.04±5.98	97.44±3.53	<0.001
NLRP3 (ng/L)	0.30±0.03	8.21±0.18	1.08±0.19	0.42±0.01	<0.001
α-Klotho (pg/mL)	562.5±15.59	154.5±15.59	470.8±15.59	520.8±15.59	<0.001

Control: healthy control group; D-Gal: induced with D-galactose only; GDF-11: growth differentiation factor-11; HP: induced with D-galactose and treated with high protein; IGF-1: insulin growth factor-1; IL-6: interleukin-6; MDA: malondialdehyde; NLRP3: NOD-like receptor family pyrin domain-containing protein 3; PG+HP: induced with D-galactose and treated with combination of porang glucomannan and high protein; SOD: superoxide dismutase; TNF-α: tumor necrosis factor-alpha

[a] Analyzed with one-way ANOVA test

Comparative analysis of oxidative stress, inflammation, and aging markers between groups

The PG+HP group showed higher mean SOD levels compared to the D-Gal and HP groups, while the D-Gal group had the lowest mean SOD levels (**Table 1** and **Figure 3A**). The levels in the PG+HP group were also the closest to those in the control group, although there was a statistically significant difference (p=0.004). The results showed that MDA levels in the PG+HP group were better than the HP and D-Gal groups (**Figure 3B**) and were closest to the control group, although there was a significant difference (p=0.003).

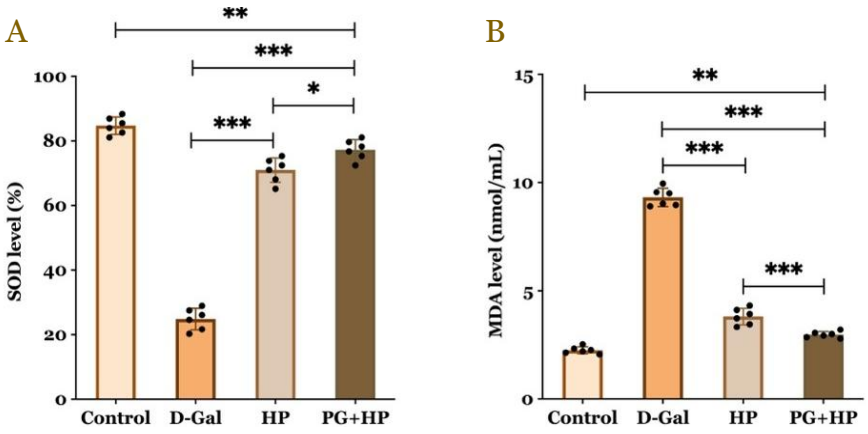

Figure 3. Effects of porang glucomannan and high protein combination diet on oxidative stress markers: (A) superoxide dismutase (SOD) and (B) malondialdehyde (MDA) levels. The measurement was performed on day 58. Control: healthy control group; D-Gal: induced with D-galactose only; HP: induced with D-galactose and treated with high protein; PG+HP: induced with D-galactose and treated with combination of porang glucomannan and high protein. Statistical analysis was performed by one-way ANOVA; significant at $^{*}p<0.05$, $^{**}p<0.01$, and $^{***}p<0.001$.

The same data trend was also presented in the inflammatory parameters, IL-6 and TNF-α levels (**Figure 4A** and **Figure 4B**). The results showed that the PG+HP group had lower IL-6 and TNF-α levels than the HP or D-Gal groups. The levels of IL-6 and TNF-α of the PG+HP group were closest to the mean levels of inflammatory markers in the normal control group. The mean levels of IL-6 and TNF-α in the D-Gal group showed the highest results.

The levels of NLRP3 and IGF-1 in the PG+HP group showed the best results compared to the HP and D-Gal groups (**Figure 5A** and **Figure 5B**). Even the NLRP3 levels of the PG+HP group were not significantly different from the control group (**Figure 5B**). The PG+HP group had the best levels of GDF-11 and α-Klotho, which were higher than the HP and D-Gal groups (**Figure 5C** and **Figure 5D**). Although there was a significant difference with the control group, the PG+HP group had the mean levels of GDF-11 and α-Klotho closest to the control group. The

results suggested that the combination of porang glucomannan and a high-protein diet could improve aging markers closely related to sarcopenia.

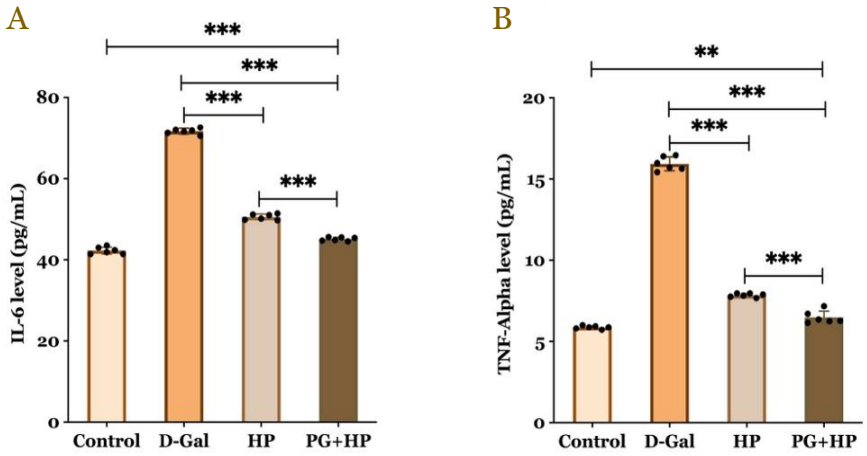

Figure 4. Effects of porang glucomannan and high protein combination diet on inflammation markers: (A) interleukin-6 (IL-6) and (B) tumor necrosis alpha (TNF-α) levels. The measurement was performed on day 58. Control: healthy control group, D-Gal: induced with D-galactose only, HP: induced with D-galactose and treated with high protein, PG+HP: induced with D-galactose and treated with combination of porang glucomannan and high protein. Statistical analysis was performed by one-way ANOVA; significant at $^{*}p<0.05$, $^{**}p<0.01$, and $^{***}p<0.001$.

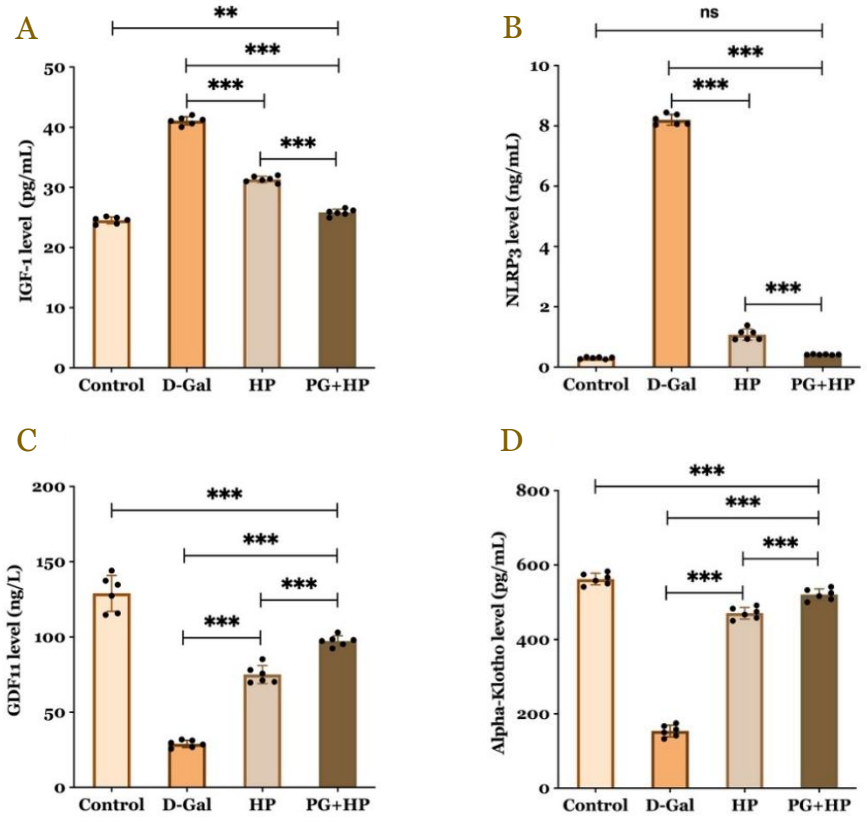

Figure 5. Effects of porang glucomannan and high protein combination diet on aging markers: insulin growth factor-1 (IGF-1) level (A); NOD-like receptor family pyrin domain-containing protein 3 (NLRP3) level (B); growth differentiation factor-11 (GDF11) (C); and α-Klotho level (D). The measurement was performed on day 58. Control: healthy control group; D-Gal: induced with D-galactose only; HP: induced with D-galactose and treated with high protein; PG+HP: induced with D-galactose and treated with a combination of porang glucomannan and high protein. Statistical analysis was performed by one-way ANOVA; ns: non-significant; significant $^{*}p<0.05$, $^{**}p<0.01$, and $^{***}p<0.001$.

Discussion

In this study, the aging induction in experimental animals was successfully achieved using an accelerated induction model with D-galactose. SOD levels in the induced groups were significantly lower compared to the control group. Previous studies using D-galactose to induce aging reported that D-galactose caused a decrease in sugar levels and essential nutrients, which reacted with amino acids to form advanced glycation end-products through non-enzymatic glycation, contributing to the formation of ROS [27,28]. These findings align with a previous study, which observed that D-galactose-induced aging resulted in increased MDA levels and decreased SOD and glutathione peroxidase levels [29]. Similar results were reported in another study, showing that D-galactose increased neuroinflammatory parameters through nuclear factor kappa B (NF-κB) activation, potentially leading to memory impairment [30].

The combination of porang glucomannan and a high-protein diet resulted in the highest SOD antioxidant levels compared to the group that received only a high-protein diet. SOD levels differed significantly between groups, with the group treated with combination of porang glucomannan and a high-protein diet having SOD levels closest to those of the control group. SOD is an enzyme that inhibits biological oxidant activity in the body, allowing it to respond to cellular oxidative stress, lipid metabolism, and inflammation [31-33]. This effect is attributed to mitochondrial damage, which leads to proton leakage and respiratory depression [31]. Additionally, a previous study emphasized that experimental rats lacking Cu/Zn SOD experienced muscle atrophy, weakness, and degeneration of the neuromuscular junction in early adulthood [34]. The results of this study align with previous research, which found that administering porang glucomannan increased SOD and glutathione peroxidase activities [35]. Another study further reinforced these findings, reporting that porang glucomannan enhanced antioxidant activity, such as SOD and MDA, reduced lipid peroxidation, and provided stomach protection [36]. Furthermore, a high-protein diet also contributes to antioxidant activity. Positive results were highlighted in a previous study on athletes, which demonstrated that a high-protein, low-carbohydrate diet increased antioxidant levels, such as SOD, and reduced inflammation [37]. Another study concluded that a chronic high-protein diet could induce excessive production of antioxidant enzymes, such as SOD and catalase, as an adaptive response to protect cells against oxidative stress [24].

Another oxidative stress marker analyzed in this study was MDA. The combination of porang glucomannan and a high-protein diet was shown to reduce oxidative stress by increasing SOD levels and decreasing MDA levels. Elevated MDA levels are common in the elderly, indicating rapid oxidation and decreased antioxidant levels in the body [38]. A previous study noted that porang contains β-D-glucose and β-D-mannose polysaccharide chains, which contribute to glucose diffusion in the intestinal lumen. However, these polysaccharides also reduce colonic MDA levels, thereby limiting oxidative reactions [38-41]. Increased MDA levels have been associated with reduced intracellular glutathione and downregulation of gut microbiota diversity, both of which trigger oxidative stress [42]. The combination of porang glucomannan and a high-protein diet was found to enhance SOD activity while reducing MDA activity, as well as the levels of TNF-α and interferon-gamma mediators involved in maintaining the intestinal mucosa [25].

Porang glucomannan combined with a high-protein diet can help reduce the inflammatory processes associated with aging, particularly those linked to intestinal microbiota dysbiosis. This study demonstrated that inflammatory parameters, such as IL-6 and TNF-α levels, were the lowest in experimental animals receiving the combination of porang glucomannan and a high-protein diet, compared to those in the high-protein only diet and D-galactose only groups. The inflammatory mediator IL-6 serves as a marker of the transition from acute to chronic inflammation, which can lead to tissue damage [43]. Additionally, TNF-α is a key regulator of the immune system during aging and is often linked to microbiota dysbiosis caused by macrophage migration [44,45]. Aging significantly alters the composition of the intestinal microbiota, leading to impaired intestinal function and an increased risk of metabolic diseases [46]. Previous studies have suggested that malnutrition in experimental animals can affect local immunity [46,47]. However, supplementation with a combination of porang glucomannan and a high-protein diet can function as a prebiotic, helping to maintain nutritional balance, modulate the intestinal microbiota, and reduce inflammation markers such as IL-6 and TNF-α [46,47].

In this study, the administration of a combination of porang glucomannan and a high-protein diet was found to influence the aging process. The study results revealed that IGF-1 hormone levels differed significantly between treatment groups, of which the group treated with a combination of porang glucomannan and a high-protein diet had lower IGF-1 levels, approaching those of the control group. These findings align with a previous study, which reported that IGF-1 is a hormone regulated by the hypothalamic-pituitary axis and plays a role in inducing aging [15]. During the aging process, IGF-1 levels are activated by PI3K and ERK/mitogen-activated protein kinase (MAPK) signaling pathways, contributing to aging in muscles and bones and increasing the risk of sarcopenia [13]. Additionally, IGF-1 regulates both life expectancy and the aging process and consequently, a reduction in IGF-1 levels is considered beneficial for extending lifespan [48,49].

Aging parameters, specifically NLRP3, also showed a lower mean level in the combination diet group, with no significant difference observed between the combination diet group and the control group. NLRP3 plays a critical role in the inflammasome process in the elderly, serving as part of the innate immune response to tissue damage and irregular inflammatory activity, such as nerve fiber damage [16,50]. Furthermore, NLRP3 inflammasome activity can trigger bone and joint damage by increasing DAMP levels, leading to elevated NLRP3 levels and contributing to sarcopenia [51,52]. Porang glucomannan, with its prebiotic effects—particularly through the promotion of lactic acid bacteria and *Bifidobacteria*—can improve intestinal microbiota composition and help reduce the incidence of sarcopenia [12]. A synergistic effect was observed between porang glucomannan and a high-protein diet, which enhanced muscle functionality and decreased the inflammasome processes underlying sarcopenia [53-55]. In contrast, a previous study has reported different results, stating that gluten-derived peptides with high glutamine and proline residue content can trigger an innate immune response in the intestine, tending to activate the NLRP3 inflammasome [54]. As a result, NLRP3 levels increased when a high-protein diet was administered alone [54].

This study found significant differences in measured GDF-11 levels between the treatment groups. The combination diet group had GDF-11 levels closer to those of the control group, although the differences remained statistically significant. These findings indicate that the combination of porang glucomannan and a high-protein diet can reduce free radical levels in the body without interfering with factors that promote tissue regeneration, such as GDF-11, and this is supported by a previous study [56]. GDF-11 is a growth factor involved in repairing organ damage and promoting neurogenesis in the spine, also known as bone morphogenic protein-11 (BMP-11), with its levels naturally decreasing with age [17,57,58]. The skeletal muscle regeneration process is also supported by porang glucomannan, as demonstrated in a previous study, which highlighted its microcarrier capabilities for regulating cell regeneration and proliferation [15]. The combined effects of porang glucomannan and a high-protein diet on regeneration and skeletogenesis can help increase GDF-11 levels [13,56]. Additionally, elderly individuals require high-protein intake to counteract the loss of muscle mass and osteoporosis associated with aging, which is partly due to the diminished anabolic response required to build new protein [59].

Another aging parameter, α-Klotho, reported in this study, showed the best results in the combination diet group compared to the group that received only a high-protein diet. The average α-Klotho levels in the combination group approached those of the control group, although significant differences were still observed between the groups [58,59]. Previous studies have shown that reduced gene expression of α-Klotho was associated with geriatric syndrome and was closely linked to higher mortality rates among older individuals and populations [60-64]. The combination of porang glucomannan and a high-protein diet demonstrated a synergistic effect in regulating blood lipids, reducing inflammatory responses, and preventing tissue damage, thereby lowering the risk of atherosclerosis [18]. Additionally, α-Klotho is known to inhibit PI3K/Akt activity, protecting the heart from oxidative stress, which may explain the increased α-Klotho levels observed with porang glucomannan supplementation [18,65-67]. A previous study also highlighted that a high-protein, low-calorie diet is among the most protective dietary approaches, as it increased serum α-Klotho and fibroblast growth factor 21 levels while serving as a neuroprotective factor [65].

A diet combining porang glucomannan and a high-protein intake, therefore, could be an ideal approach because it has many roles in the aging mechanism in the elderly. Porang glucomannan, as a prebiotic, can reduce oxidative stress by enhancing antioxidant activity [66,67]. Meanwhile, a high-protein diet is considered highly beneficial for older adults because it helps prevent inflammation and oxidative stress in skeletal muscles and bones [68]. The prebiotic effects of porang glucomannan improve intestinal microbiota composition and regulate muscle functionality by modulating systemic and tissue inflammation. Probiotics derived from lactic acid bacteria and *Bifidobacteria* have been shown to prevent the incidence of sarcopenia [12], a degenerative condition characterized by the loss of skeletal muscle mass and function [53]. High protein intake is essential for building muscle mass, preventing sarcopenia, and promoting healthy aging [69]. The combination of porang glucomannan as a prebiotic and a high-protein diet works synergistically as an effective anti-aging strategy. However, further research is needed to determine the optimal dosage, safety, and preparation methods for this combination.

Conclusion

The combination of porang glucomannan and a high-protein diet was superior in improving various aging markers, including oxidative stress markers (SOD and MDA), inflammatory markers (TNF-α and IL-6), and aging markers (IGF-1, GDF-11, NLRP3, and α-Klotho). These results suggested that this diet combination could be an effective anti-aging intervention. Further investigation is needed to assess its safety and the formulation of the combination's preparation.

Ethics approval

Approval for the study was obtained from the Research Bioethics Committee, Faculty of Medicine, Universitas Sultan Agung Islamic, Semarang, Central Java, Indonesia (No.301/VIII/202/Bioethics Committee).

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None to be declared.

Competing interests

All the authors declare that there are no conflicts of interest.

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Underlying data

Derived data supporting the findings of this study were available from the corresponding author on request.

Declaration of artificial intelligence use

We hereby confirm that no artificial intelligence (AI) tools or methodologies were utilized at any stage of this study, including during data collection, analysis, visualization, or manuscript preparation. All work presented in this study was conducted manually by the authors without the assistance of AI-based tools or systems.

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