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6-Shogaol Attenuates Renal Aging Induced by D-galactose via Inhibiting PI3K/AKT/FoxO3 Signaling-mediated Autophagy

Yuehan Zhu[1,2,a,†], Ziqi Chen[1,3,b,†], Jiansheng Cai[1,2,c], Tingyu Mai[1,2,d], Chaofan Xie[1,2,e], Bin Wang[1,2,f], You Li[1,2,g,*]

[1]Department of Environmental Health and Occupational Medicine, School of Public Health, Guilin Medical University, Guilin, 541199, China
[2]Guangxi Health Commission Key Laboratory of Entire Lifecycle Health and Care (Guilin Medical University), Guilin, 541199, China
[3]Qinzhou Center for Disease Control and Prevention, Qinzhou, 535000, China
[a]2539174749@qq.com, [b]chenziqi8129@163.com, [c]15007714226@163.com, [d]owlmaiTY@outlook.com, [e]xiechaofan0226@163.com, [f]15390980068@163.com, [g]liyou121300@163.com
†These authors contributed equally to this work
**Corresponding author*

Abstract: *This study aimed to investigate whether the protective effect of 6-shogaol on aging-induced renal injury is associated with autophagy mediated by the PI3K/AKT/FoxO3 signaling pathway. The aging mouse model was established by intraperitoneal injection of D-galactose (D-gal), followed by intragastric administration of 6-shogaol at low, medium and high doses (5, 10, 20 mg/kg body weight). We measured serum creatinine (CRE) and blood urea nitrogen(BUN) levels. We examined renal histopathology and aging status using hematoxylin-eosin (H&E) staining, Masson's trichrome staining, and β-galactosidase staining. We determined superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and malondialdehyde (MDA) levels in renal tissue. The mRNA expression levels of Beclin1, LC3, Cdkn1a, PI3K, AKT, and FoxO3 were quantified by quantitative real-time PCR (qRT-PCR). We assessed the expression of autophagy marker proteins Beclin1 and LC3B using Immunohistochemistry(IHC). We also evaluated PI3K/AKT/FoxO3 pathway-related protein expression and phosphorylation status. Compared with the control group, the D-gal group displayed reduced organ coefficients($P<0.01$), increased serum BUN and CRE levels($P<0.05$), severe renal histopathological injury and collagen deposition, accelerated aging phenotypes, decreased SOD and GSH-Px activities($P>0.05$), elevated MDA content($P<0.05$), together with upregulated mRNA levels of Beclin1, LC3, Cdkn1a, AKT and FoxO3 and enhanced protein expression of the autophagy markers Beclin1 and LC3B($P<0.05$), downregulated p-AKT/AKT($P<0.05$). When compared with the D-gal model group, 6-shogaol intervention decreased serum BUN and CRE levels in a dose-dependent manner, with the medium-dose group showing the optimal therapeutic effect($P<0.05$). Meanwhile, 6-shogaol ameliorated renal histopathological damage, elevated SOD and GSH-Px activities, and lowered MDA content($P<0.01$). Furthermore, it downregulated the mRNA levels of LC3, Cdkn1a, PI3K and FoxO3 as well as the protein expression of autophagy-related markers Beclin1 and LC3B($P<0.05$), while concurrently increasing the phosphorylation of PI3K/AKT/FoxO3 pathway proteins($P<0.05$). Collectively, these findings demonstrate that 6-shogaol can alleviate aging-induced renal injury, and its renoprotective mechanism may involve the inhibition of excessive autophagy via modulating the PI3K/AKT/FoxO3 signaling pathway.*

Keywords: *6-Shogaol, D-galactose, Kidney aging, Autophagy*

1. Introduction

Global population aging has emerged as a formidable public health challenge, and this demographic transition has become a pressing societal and medical issue for China in the 21st century[1]. The health burden attributable to population aging and its attendant comorbidities continues to rise globally[2]. Advanced age triggers progressive functional deterioration of vital organs including the heart, brain, liver, and kidneys, which constitutes a core pathological basis for age-associated disorders[3]. Among these organs, the kidney is particularly vulnerable to aging-related damage. Since 1990, the global

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incidence and mortality of chronic kidney disease (CKD) have risen steadily, and CKD is predicted to rank as the fifth leading cause of death worldwide by 2040[4]. While chronological aging alone does not necessarily induce overt renal pathological lesions, the progressive accumulation of aging-related risk factors drives the initiation and progression of diverse renal diseases[5]. Cumulative renal damage induced by aging compromises renal functional reserve and lowers renal tolerance to stress-mediated decompensation, thereby increasing the susceptibility to renal insufficiency and elevating mortality in elderly individuals.Currently, the clinical management of aging-associated kidney diseases predominantly relies on pharmacological interventions, which are limited by suboptimal therapeutic efficacy and unavoidable adverse side effects. Accordingly, screening and identifying safe and efficacious natural bioactive compounds has become a promising research direction for the prevention and treatment of renal aging.

6-Shogaol, a primary bioactive component derived from ginger, possesses multiple pharmacological properties, including anti-tumor, antioxidant, anti-inflammatory, and anti-aging activities[6,7]. Accumulating preclinical evidence has validated the renoprotective effects of 6-shogaol in diabetic nephropathy animal models through multiple regulatory mechanisms. Specifically, 6-shogaol improved renal function by reducing serum urea and creatinine levels, alleviates renal oxidative stress and hyperglycemia, and suppresses renal inflammatory responses[8]. Moreover, a previous study demonstrated that 6-Shogaol could ameliorate age-related cognitive deficits in aged mice by enriching butyrate-producing gut microbiota and enhancing intestinal barrier integrity[9]. Nevertheless, whether 6-shogaol exerts protective effects against renal aging remains poorly elucidated, and the underlying mechanisms require further investigation.

Oxidative stress, inflammatory responses, and dysregulated autophagy are well-recognized core mechanisms driving renal aging and aging-associated renal injury, with oxidative stress serving as a central pathogenic contributor[8]. Forkhead box O3 (FoxO3), a pivotal member of the Forkhead box O transcription factor family[10], modulates a spectrum of cellular biological processes, including oxidative stress responses, autophagy, and apoptosis, and actes as a master regulator of cellular redox homeostasis[11]. Mitochondrial reactive oxygen species (ROS) have been confirmed to negatively regulate FoxO3 expression[12]. Under renal stress and pathological injury conditions, FoxO3 activation can exert prominent renoprotective effects primarily by transcriptionally activating downstream antioxidant genes[11]. In addition, FoxO3 is a critical upstream regulator of autophagy during renal damage, which promoted autophagy activation by upregulating the transcription of core autophagy-related genes such as LC3B and Beclin1[13]. Autophagy is an essential cellular catabolic pathway that maintains renal tissue homeostasis during aging, and its activity is precisely modulated by multiple signaling cascades, including the PI3K/Akt, AMPK/mTOR, and Sirt1-FoxO3 signaling axes[14–17]. Notably, both insufficient and excessive autophagy contribute to abnormal renal aging and pathological damage[8]. The PI3K/Akt signaling pathway is a key regulatory cascade governing cellular homeostasis and the aging process[18–20], and FoxO3 serves as a vital downstream transcriptional effector that tightly links PI3K/Akt signaling to autophagy regulation[21]. On the basis of the above research findings, the present study established a D-galactose-induced renal aging mouse model to explore the regulatory effects of 6-shogaol on renal autophagy during aging and clarify whether its renoprotective function was mediated via the PI3K/Akt/FoxO3 signaling pathway. This study aimes to provide novel mechanistic insights and a theoretical basis for the application of natural compounds in delaying renal aging.

2. Materials and Methods

2.1 Experimental animals

Specific-pathogen-free (SPF) male C57BL/6 mice (6 weeks old, body weight: 21±2 g) were purchased from Hunan Sleek Jingda Laboratory Animal Co., Ltd. (License No.: SCXK(Xiang) 2016-0002). A total of 72 mice were included in this study. All animals were reared in the SPF barrier facility of the Animal Experimental Center of Guilin Medical University under standardized housing conditions: a 12 h light/dark cycle, constant ambient temperature of 22 ± 2 °C, relative humidity of 40% to 70%, and ad libitum access to standard laboratory chow and drinking water. Clean and well-ventilated cage environments were consistently maintained during the entire feeding period. All animal experimental protocols were reviewed and approved by the Institutional Animal Care and Use Committee of Guilin Medical University (Approval No. GLMC-IACUC-2021026), and all animal

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handling and experiments were performed in strict accordance with relevant institutional and national guidelines.

2.2 Main reagents and instruments

6-Shogaol (≥98%), Chengdu Pufei De Biotech Co., Ltd.; Rapamycin (RAPA, ≥99%), MCE; D-galactose; phosphate-buffered saline ; Hematoxylin-eosin (H&E) staining kit; Masson's trichrome staining kit; β-galactosidase (SA-β-gal) staining kit, Beijing Solarbio Science & Technology Co., Ltd.; BCA protein concentration assay kit (enhanced type), Shanghai Beyotime Biotechnology Co., Ltd.; Blood urea nitrogen (BUN) assay kit, serum creatinine (CRE) assay kit, total superoxide dismutase (SOD) assay kit (WST-1 method), glutathione peroxidase (GSH-Px) assay kit, malondialdehyde (MDA) assay kit, Nanjing Jiancheng Bioengineering Institute; Immunostaining blocking solution/primary antibody diluent, DAB immunohistochemistry color development kit, BBI; Omni-ECL™ ultra-sensitive chemiluminescence detection kit, Shanghai Epizyme Biomedical Technology Co., Ltd.; PAGE color (red, 8%) gel ultra-rapid preparation kit, Wuhan Servicebio Technology Co., Ltd.; HRP-labeled mouse secondary antibody and rabbit secondary antibody, Shanghai Changdao; Horseradish peroxidase-labeled goat anti-rabbit IgG antibody/anti-mouse IgG antibody, rabbit anti-LC3-II antibody/anti-Beclin1 antibody and anti-p-Akt(Ser473) antibody and anti-FoxO3 antibody and anti-p-FoxO3(Ser315) antibody, mouse anti-Akt antibody and anti-GAPDH antibody, Wuhan Proteintech Group, Inc.

Slide scanner, Olympus, Japan; Real-time PCR system, ABI, USA; Multicolor fluorescence, chemiluminescence, and visible light fully automatic imaging system, ProteinSimple, USA.

2.3 Animal grouping and administration

Seventy-two 6-week-old male C57BL/6 mice were acclimated for 1 week and then randomly assigned to 6 groups (n = 12 per group). Control group: intragastric administration of 0.01 mL/g vehicle followed by intraperitoneal injection of an equal volume of PBS; D-gal group: intragastric administration of an equal volume of vehicle and intraperitoneal injection of D-galactose (200 mg/kg/day)[20]; Low-dose 6-shogaol group: intraperitoneal injection of an equal volume of D-galactose and intragastric administration of 6-shogaol (5 mg/kg/day); Medium-dose 6-shogaol group: intraperitoneal injection of an equal volume of D-galactose and intragastric administration of 6-shogaol (10 mg/kg/day); High-dose 6-shogaol group: intraperitoneal injection of an equal volume of D-galactose and intragastric administration of 6-shogaol (20 mg/kg/day); RAPA group: intraperitoneal injection of an equal volume of D-galactose and simultaneous injection of rapamycin (2 mg/kg/day). The doses of 6-shogaol were based on studies by Cui and Zhang et al.[22,23]. All groups were treated according to the experimental protocol for 42 days.

2.4 Determination of mouse organ coefficients

$$Organ\ coefficient\ (mg/g) = organ\ weight\ (mg) / mouse\ body\ weight\ (g) \quad (1)$$

2.5 Determination of renal function indicators

Serum BUN and CRE levels were measured using commercial assay kits according to the manufacturer's protocols.

2.6 Observation of renal tissue pathological structure

Renal tissues were collected, fixed in 4% paraformaldehyde, embedded, sectioned, and deparaffinized. H&E staining, Masson's trichrome staining, and SA-β-gal staining were performed strictly according to the kit instructions. After drying and mounting, the sections were scanned, photographed, and stored.

2.7 Detection of renal tissue oxidative stress levels

The activities of SOD and GSH-Px and the level of MDA were determined according to the kit instructions.

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2.8 qRT-PCR detection of target gene expression

Renal tissue samples were ground into homogenates. Total RNA was extracted with the Trizol method and reverse-transcribed into cDNA. The qPCR reaction program was: 95 °C for 10 s, followed by 40 cycles of 95 °C for 5 s and 60 °C for 30 s. Relative mRNA expression levels were calculated using the 2^(-ΔΔCt) method. The primer sequences for the target genes were listed in Table1.

Table 1: qRT-PCR related gene primer sequences.

Gene	FORWARD	REVERSE
Cdkn1a	TCCTGGTGATGTCCGACCTGTTC	CCCTCCAGCGGCGTCTCC
FoxO3	CACACTACGGCAACCAGACACTC	TGGTGGTGGAGCAAGTTCTGATTG
Beclin1	CAGTACCAGCGGGAGTATAGTGAG	TGGAAGGTGGCATTGAAGACATTG
LC3	GAGCGAGTTGGTCAAGATCATCC	TCCGTCTTCATCCTTCTCCTGTTC
PI3K	TATGTCTACCCTCCAAATGTCG	TACTTCTGCTTGTCGTTGTTG
AKT	TGCACAAACGAGGGGAATATAT	CGTTCCTTGTAGCCAATAAAGG
GAPDH	AGGCTGTGGGCAAGGTCATC	TGTCATCATACTTGGCAGGTTTCTC

2.9 Immunohistochemical staining for Beclin1 and LC3B proteins

Renal tissue sections were subjected to antigen retrieval in trisodium citrate buffer, blocked with blocking buffer for 45 min, and incubated with diluted primary antibody overnight at 4°C. Subsequently, ready-to-use enzyme-labeled secondary antibody was added and incubated for 45 min at room temperature. DAB color development was performed, followed by mounting, imaging, and storage.

2.10 Western blot detection of AKT/p-AKT and FoxO3/p-FoxO3 protein expression

Renal tissues were lysed and centrifuged to collect supernatants. Protein concentrations were measured by BCA assay. Proteins were separated by SDS-PAGE and transferred to membranes, followed by routine blocking. The membranes were incubated with primary antibody overnight at 4 °C, and then with the corresponding secondary antibody for 1h at room temperature. After chemiluminescent detection, the band intensities were quantified using ImageJ software.

2.11 Data processing

All data were expressed as the mean ± standard deviation. Statistical analyses were performed using SPSS 28.0. Group differences were assessed by one-way analysis of variance (ANOVA), with post hoc multiple comparisons performd using the least significant difference (LSD) test. A two-tailed $P < 0.05$ was considered statistically significant.

3. Results

3.1 Effects of 6-shogaol on organ index and renal function in aging mice

Table 2: 6-shogaol on renal organ coefficients, BUN and CRE content in aging mice($\bar{x} \pm S$, N=9).

Group	Organ index(mg/g)	BUN(mmol/L)	CRE(μmol/L)
Control	11.82±0.18	8.028±0.688	21.72±4.71
D-gal	10.34±0.43$^{\#\#}$	9.829±0.847$^{\#}$	40.95±9.67$^{\#\#}$
Low-dose-6-shogaol	10.73±0.13$^{\#\#}$	8.298±0.553*	28.07±3.12
Medium-dose-6-shogaol	11.07±0.46$^{\#\#**}$	7.417±0.944**	22.75±3.51**
High-dose-6-shogaol	10.77±0.31$^{\#\#}$	9.213±0.882	29.31±5.78*
RAPA	11.76±0.27**	8.181±0.156*	23.08±1.78**
P	<0.0001	0.026	0.008

Note: Compared with control group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$; compared with D-gal group, $*P < 0.05$, $**P < 0.01$. Same for Tables 2-4.

Compared with the control group, the kidney index was significantly decreased in all treatment groups (P <0.01), with the most pronounced reduction observed in the D-gal group. Compared with the D-gal group, all 6-shogaol-treated groups exhibited increased kidney index. However, only the

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medium-dose group and the RAPA group reached statistical significance ($P < 0.01$). Serum BUN and CRE levels were markedly higher in the D-gal group than in the control group ($P < 0.05$). Compared with the D-gal group, the lowest BUN and CRE levels were observed in the medium-dose 6-shogaol group ($P < 0.05$), and similar reductions were observed in the RAPA group ($P < 0.05$). Detailed results were presented in Table 2.

3.2 Effects of 6-Shogaol on Renal Pathology and Senescence in Aging Mice

3.2.1 H&E staining

As shown in Figure 1, the control group showed intact renal structure with normal glomerular and tubular morphology. In contrast, the D-gal group exhibited severe renal pathological injuries, including dilated Bowman's spaces, shrunken glomeruli, narrowed tubular lumens, and disorganized renal tissue. 6-Shogaol treatment alleviated these histological abnormalities, with the medium-dose group showing the best improvement. RAPA intervention also effectively rescued D-gal-induced renal damage.

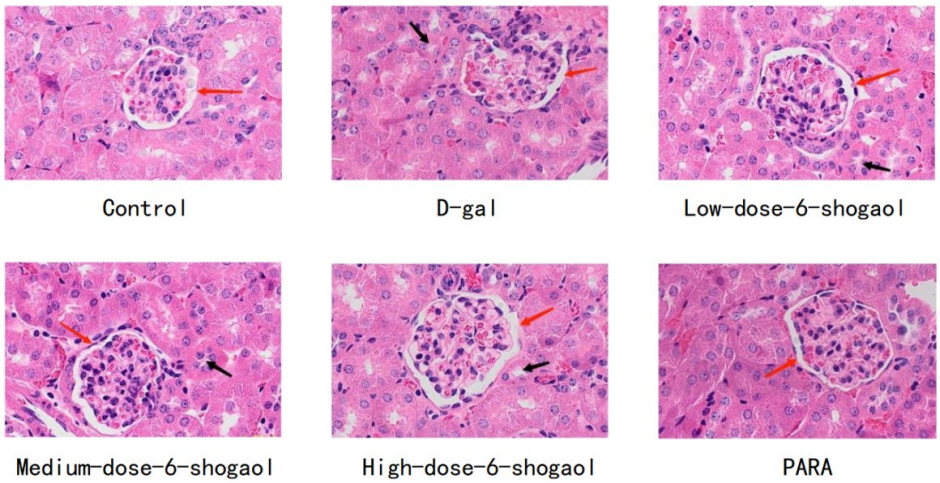


Figure 1: Effect of 6-shogaol on the pathological structure of the kidney in aging mice (HE staining,20×).

3.2.2 Masson's trichrome staining

The control group exhibited sparse positive staining in the renal interstitium. The D-gal group displayed markedly increased positive staining area and intensity. 6-Shogaol treatment reduced positive staining in a dose-dependent manner, with the most significant improvement observed in the medium-dose group. Notably, the RAPA group showed minimal positive staining. These staining changes were illustrated in Figure 2.

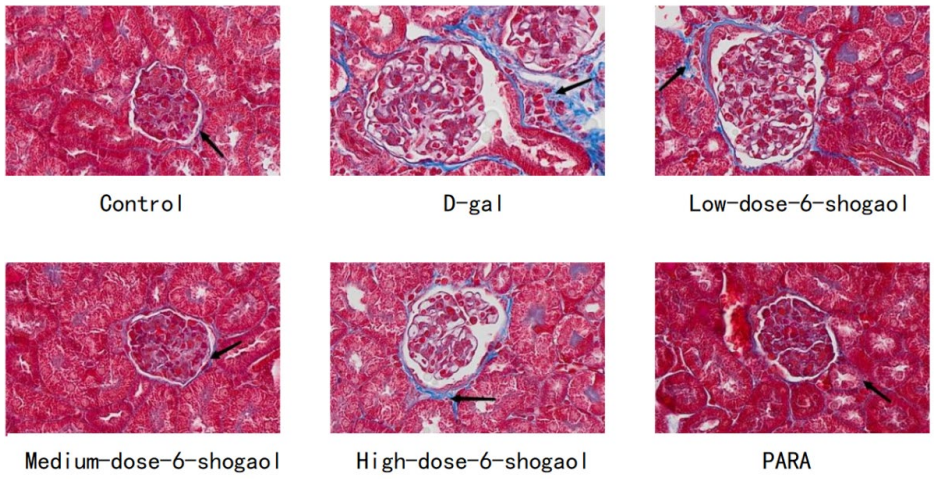


Figure 2: Effect of 6-shogaol on fibrotic changes in renal tissues of aging mice (Masson staining, 20×).

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3.2.3 β-Galactosidase staining

As shown in Figure 3, the D-gal group showed a markedly larger SA-β-gal-positive area and deeper blue staining than the control group. 6-Shogaol treatment reduced the stained area in all dose groups, particularly in the medium- and high-dose groups, whereas only scattered blue granules were observed in the RAPA group.

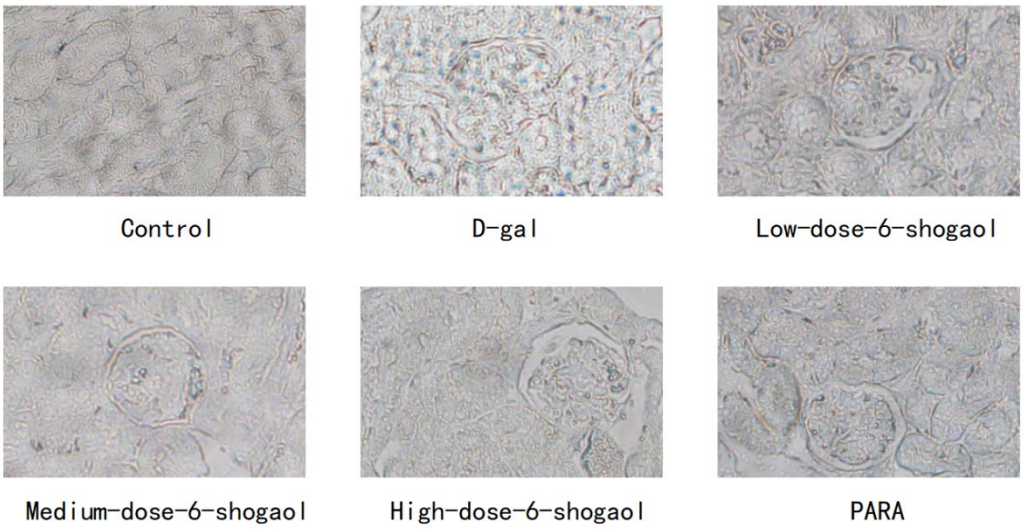


Figure 3: Effect of 6-shogaol on β-galactosidase staining of renal tissues in aging mice (20×).

3.3 Effects of 6-shogaol on renal oxidative stress markers in aging mice

As shown in Table 3, no significant difference in renal SOD activity was observed among all groups ($P > 0.05$). GSH-Px activity did not differ significantly between the control and D-gal groups ($P > 0.05$); however, it was markedly elevated in the medium-dose, high-dose, and RAPA groups compared with the D-gal group ($P < 0.01$). In contrast, renal MDA content in the D-gal group was increased by 99.14% relative to the control group ($P < 0.05$), and was significantly decreased in the medium-dose, high-dose, and RAPA groups compared with the D-gal group ($P < 0.01$).

Table 3 : Effect of 6-shogaol on renal SOD, GSH-Px, and MDA contents in aging mice($\bar{x} \pm S$, N=9).

Group	SOD(U/mgProt)	GSH-Px(mol/mgProt)	MDA(nmol/mgProt)
Control	20.40±5.21	31.12±7.55	1.52±0.68
D-gal	13.84±1.23	20.60±4.43	$3.03\pm0.99^{\#}$
Low-dose-6-shogaol	16.79±8.81	21.16±4.62	$3.64\pm0.57^{\#\#}$
Medium-dose-6-shogaol	18.18±3.48	$84.00\pm10.99^{\#\#**}$	$1.05\pm0.09^{**}$
High-dose-6-shogaol	13.58±1.68	$62.53\pm11.95^{\#\#**}$	$1.58\pm0.17^{*}$
RAPA	15.09±4.24	$63.82\pm7.63^{\#\#**}$	$1.51\pm0.23^{*}$
P	0.686	<0.0001	0.003

3.4 Effects of 6-Shogaol on Autophagy- and PI3K/AKT/FoxO3-Related Gene Expression in Aging Mouse Kidneys

Quantitative PCR analysis (Figure 4) revealed that the D-gal group had significantly higher mRNA levels of *Beclin1, LC3, Cdkn1a, AKT*, and *FoxO3* than the control group ($P < 0.05$). In contrast, treatment with 6-shogaol substantially reduced the transcript levels of *LC3*, *Cdkn1a*, *PI3K*, and *FoxO3* relative to the D-gal group ($P < 0.05$).

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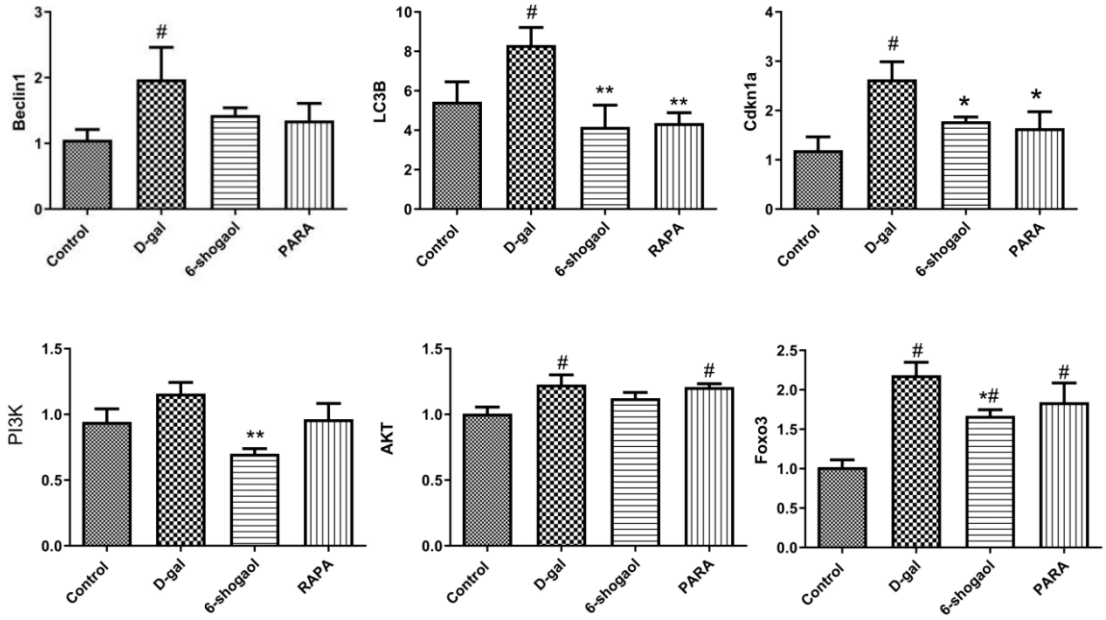


Figure 4: Effects of 6-shogaol on the expression of Beclin1, LC3, Cdkn1a, PI3K, AKT and FoxO3 genes in renal tissues of aging mice.

3.5 Effects of 6-Shogaol on Renal Beclin1 and LC3B Protein Expression in Aging Mice

In renal tissue sections, positive immunostaining for Beclin1 and LC3B appeared as brown deposits. The staining intensity was markedly stronger in the D-gal group than in the control group, indicating significant upregulation of both proteins ($P < 0.05$). In contrast, 6-shogaol treatment resulted in visibly weaker staining and reduced immunostaining intensity compared with the D-gal group ($P < 0.01$) (Figures 5 and 6).

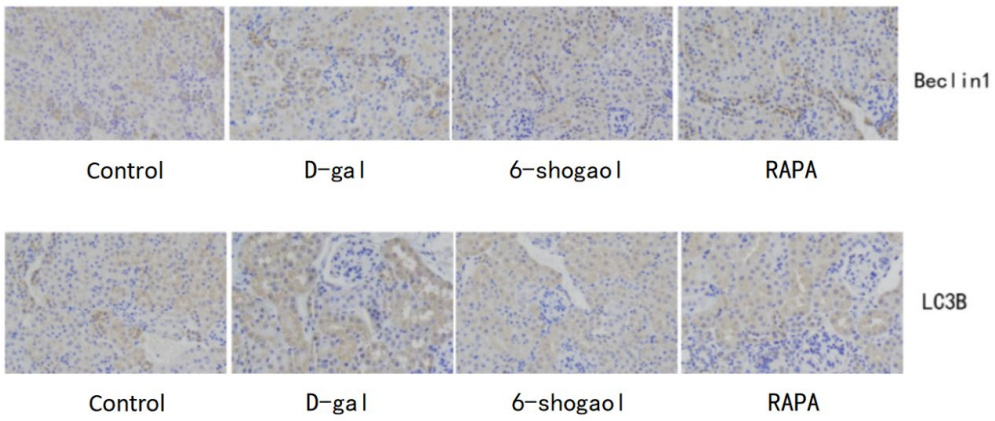


Figure 5: Effect of 6-shogaol on the expression of Beclin1 and LC3B in renal tissues of aging mice (10×).

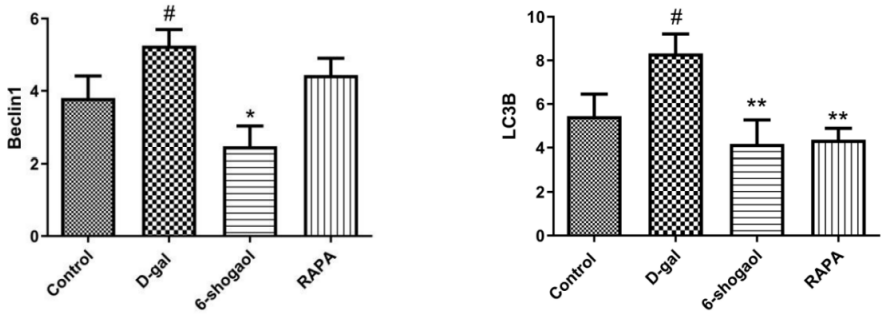


Figure 6: Effect of 6-shogaol on the expression of Beclin1 and LC3B in renal tissues of aging mice.

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3.6 Effects of 6-Shogaol on Renal AKT, p-AKT, FoxO3, and p-FoxO3 Protein Expression in Aging Mice

As shown in Table 4 and Figure 7, total AKT, total FoxO3, p-AKT, and p-FoxO3 levels were comparable between the D-gal and control groups ($P > 0.05$), the p-AKT/AKT ratio was lower in the D-gal group than in the control group ($P < 0.05$). 6-Shogaol treatment significantly increased p-AKT and p-FoxO3 levels, as well as both phosphorylation ratios ($P < 0.05$).

Table 4: Effects of 6-shogaol on AKT, p-AKT, FoxO3, p-FoxO3 protein expression in renal tissues of aging mice ($\bar{x} \pm S$).

Group	AKT/GAPDH	p-AKT/GAPDH	FoxO3/GAPDH	p-FoxO3/GAPDH
Control	0.37±0.07	0.49±0.14	1.11±0.19	0.11±0.02
D-gal	0.63±0.23	0.44±0.06	1.28±0.21	0.09±0.4
6-shogaol	0.43±0.07	0.65±0.13*	1.15±0.07	0.26±0.05$^{\#*}$
RAPA	0.55±0.16	0.30±0.05$^{\#}$	0.78±0.11$^{\#*}$	0.16±0.04*
P	0.202	0.017	0.025	0.003

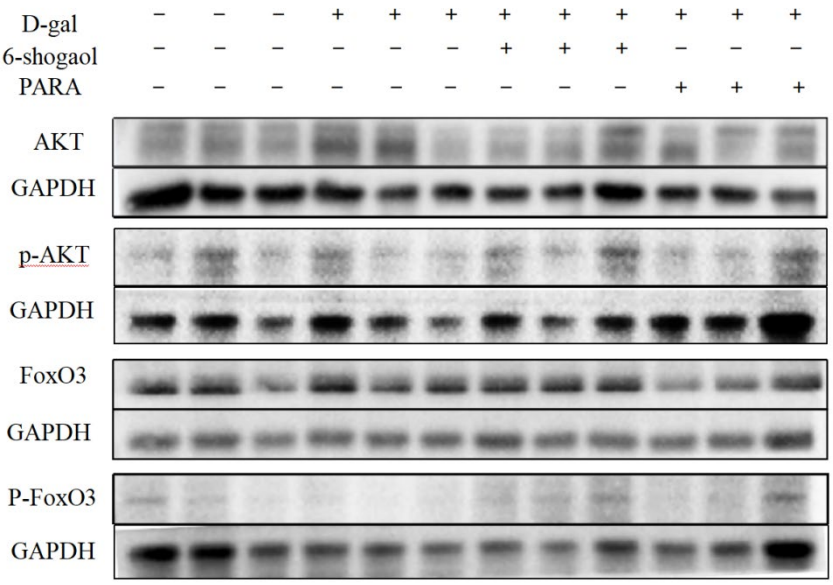


Figure 7: Effects of 6-shogaol on AKT, p-AKT, FoxO3, p-FoxO3 protein expression in renal tissues of aging mice.

4. Discussion

The D-gal group exhibited decreased kidney index and elevated serum CRE and BUN levels, suggesting renal atrophy and functional impairment in this model. Following 6-shogaol treatment, kidney index was restored and CRE and BUN levels were reduced compared with the D-gal group. The RAPA group showed a comparable improvement. These results indicated that 6-shogaol could attenuate D-galactose-induced renal atrophy and facilitate recovery of kidney metabolic function. Rapamycin exhibited a similar effect. These findings were consistent with those of Ren et al.[24], who reported that *Dendrobium officinale* extract ameliorates aging-induced kidney injury. Studies have demonstrated that 6-shogaol exerts antioxidant effects in diabetic mice[27], and delayed vascular aging through the mTOR signaling pathway[7]. This study further demonstrated that 6-shogaol can enhance renal function and confer protective effects against age-related kidney injury.

H&E and Masson trichrome staining revealed that the D-gal group exhibited loose glomerular architecture with reduced glomerular volume and dilated Bowman's spaces. Renal tubular epithelial cells appeared hypertrophic with narrowed lumens, and focal areas showed atrophic changes. Marked interstitial collagen deposition was evident as intense blue staining. Following 6-shogaol treatment, these pathological alterations were ameliorated: glomerular structure became more regular, Bowman's spaces contracted, and tubular morphology tended toward normalization with reduced interstitial collagen fibers. The RAPA group displayed renal corpuscle and tubular morphology comparable to the

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medium-dose group, with minimal collagen deposition. Collectively, these findings demonstrated that 6-shogaol reversed D-galactose-induced renal senescence and attenuated fibrotic changes. Previous studies have demonstrated that alginate oligosaccharides attenuate collagen deposition and alleviate pathological injury in D-galactose-induced renal aging; by analogy, 6-shogaol may exert a comparable protective mechanism[8]. SA-β-gal staining revealed extensive, intensely the blue-stained deposits throughout the renal tissue of the D-gal group, indicative of a pronounced senescent phenotype, consistent with previous reports[25]. Following 6-shogaol treatment, blue-stained area was reduced across all dose groups, with the most pronounced decrease observed in the medium- and high-dose groups, suggesting that 6-shogaol exerted anti-aging effects.

Oxidative stress injury is recognized as one of the key pathogenic factors in various kidney diseases, including chronic renal failure[26], acute kidney injury[27], and diabetic nephropathy[28]. The D-gal group exhibited reduced SOD and GSH-Px activities and elevated MDA levels relative to controls, indicating that the kidneys had undergone oxidative damage. Following 6-shogaol treatment, GSH-Px activity was restored and MDA levels were reduced in the medium- and high-dose groups compared with the D-gal group, with the most pronounced improvement observed in the medium-dose group. These results suggest that 6-shogaol alleviates lipid peroxidation in renal tissue, possibly by enhancing local antioxidant defenses. Gwon et al.[29]reported that 6-shogaol alleviates oxidative damage and protects against acute kidney injury in mice. Another study demonstrated that 6-shogaol protects against kidney damage in diabetic models by suppressing oxidative stress[30]. As a bioactive constituent of Zingiberaceae plants, 6-shogaol may exert antioxidant effects in the aging kidney comparable to those of curcumin[31]. Excessive ROS generation in renal aging triggers oxidative stress, resulting in damage to DNA, proteins, and phospholipids, and ultimately tissue injury[32]. Collectively, these findings suggested that the renoprotective effect of 6-shogaol may be mediated through modulation of ROS levels.

qRT-PCR and immunohistochemistry revealed that D-galactose upregulated the expression of autophagy markers and *Cdkn1a* in the aging kidney. Following 6-shogaol treatment, the expression of *Beclin1*, *Cdkn1a*, and *LC3* was downregulated, autophagy was attenuated, and aging-related renal damage showed partial improvement. Studies have demonstrated that Cdkn1a mediates cell cycle arrest through CDK inhibition upon telomeric DNA damage, thereby facilitating DNA repair and restoring cellular homeostasis[33]. However, excessive *Cdkn1a* expression induces cell cycle arrest and a senescent phenotype[34]. The altered expression of *Beclin1* and *LC3B* suggested that 6-shogaol may ameliorate kidney injury through autophagy inhibition. This finding was consistent with the report by Qi et al.[15] that cinnamaldehyde suppresses autophagy via the PI3K/AKT pathway, thereby attenuating renal aging injury.

Western blot analysis revealed that the p-AKT/AKT ratio was decreased in the D-gal group compared with the control. Following treatment with 6-shogaol, p-AKT and p-FoxO3 levels increased, as did the p-AKT/AKT and p-FoxO3/FoxO3 ratios. These findings suggested that 6-shogaol activated the PI3K/AKT pathway and enhanced FoxO3 phosphorylation. Li et al.[13] demonstrated in a unilateral ureteral obstruction (UUO) mouse model that hypoxia-induced FoxO3 accumulation activated autophagy, which, when sustained in renal tubular epithelial cells, accelerated interstitial fibrosis. Collectively, 6-shogaol ameliorated D-galactose-induced renal aging and injury by improving renal metabolism, attenuating pathological damage and fibrosis, reducing oxidative stress, and modulating autophagy and senescence-associated gene expression. These protective effects were mediated, at least in part, through the PI3K/AKT/FoxO3 axis-dependent regulation of autophagy.

5. Conclusion

In summary, 6-shogaol protects against aging-associated renal injury, likely through suppression of autophagy via modulation of the PI3K/AKT/FoxO3 signaling pathway. These findings can provide experimental evidence supporting the development of therapeutic strategies for aging-related kidney diseases.

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