

Renal function-improving action of Pentadecyl[®], a multifunctional triglyceride from *Aurantiochytrium limacinum*, in a mouse model of chronic kidney disease

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Abstract. Pentadecyl[®], derived from *Aurantiochytrium limacinum*, is a triglyceride primarily composed of odd-numbered fatty acids such as pentadecanoic acid (C15). Pentadecyl[®] improves glucose tolerance in a mouse model of type II diabetes and promotes collagen production in human skin cells. However, its effects on kidney failure and chronic kidney disease (CKD) remain unexplored. The present study examined the effects of continuous Pentadecyl[®] administration on renal dysfunction in 5/6-nephrectomized mice (5/6Nx mice), a well-established model of CKD. Pentadecyl[®] (0.2 g/l) was administered orally *via* a suspension in drinking water for 8 weeks. Although survival analysis using the log-rank test revealed no significant differences, a significant improvement in body weight gain was observed in 5/6Nx mice treated with Pentadecyl[®] compared with that in untreated controls, despite similar food and water intake. Renal function was assessed by measuring blood urea nitrogen (BUN) and serum creatinine (CRE) levels, both of which were significantly elevated in 5/6Nx mice. Notably, Pentadecyl[®] administration attenuated the increase in BUN and CRE levels. Histopathological analysis through hematoxylin and eosin staining revealed characteristic changes in the kidneys of 5/6Nx mice, including glomerular hypertrophy and tubular dilation. There was no significant difference in the mean glomerular and tubular areas between Pentadecyl[®]-treated mice and 5/6Nx mice. Nonetheless, distribution analyses descriptively showed

fewer enlarged tubular profiles. These findings suggest that Pentadecyl[®] exerts protective effects against renal dysfunction in CKD, potentially contributing to the prevention of uremic complications.

Introduction

Chronic kidney disease (CKD) is a clinical syndrome characterized by a persistent loss of renal function over months or years. The diagnostic criteria are established when either or both a decrease in the glomerular filtration rate and confirmation of markers of renal impairment (e.g., abnormal tissue, albuminuria, and electrolyte abnormalities) have persisted for at least 3 months (1). The increasing number of patients with CKD is a growing global health concern associated with the aging population and the increasing prevalence of diabetes mellitus and hypertension. Renal injury in patients with CKD has been attributed to many potential mechanisms, including age-related renal tubular cell death (2), kidney fibrosis (3), chronic hypoxic stress and endoplasmic reticulum (ER) stress (4), oxidative stress, and chronic inflammation (5), with the exact mechanism being unclear. In most cases, as CKD progresses to end-stage renal disease, uremia develops, with symptoms such as fluid abnormalities, gastrointestinal problems, anemia, and impaired consciousness (6). The cause is the impaired excretion of endogenous metabolites in the urine, resulting in the accumulation of high concentrations of uremic substances in the body (7). In particular, uremia has a significant impact on survival because it causes multi-organ diseases; however, the detailed mechanisms also remain unclear. Recent studies have focused on preventing and halting the progression of CKD to end-stage renal disease. Therefore, there is an urgent need to develop safer and more effective treatments to manage and prevent uremia in patients with CKD.

Experimental animal models that have been used for the purpose of studying the pathogenesis and development of drugs in CKD include the adenine diet model (8), 5/6-nephrectomy (5/6Nx) model (9), and nephrectomy plus ischemia-reperfusion injury model (10). Among them, the 5/6Nx mouse model, in which 2/3rd of the left kidney is removed and the right kidney

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is completely removed, is referred to as a subtotal nephrectomy model and is the most widely used because it reproduces the features of patients with CKD, including elevated serum creatinine (CRE) and blood urea nitrogen (BUN) levels (9). In addition to azotemia, this model is accompanied by accumulation of uremic solutes such as indoxyl sulfate and p-cresyl sulfate (11). Renal injury in 5/6Nx mice involves inflammatory responses (12), oxidative stress (13), mitochondrial dysfunction (14,15), and ER stress activation (15), and interventions targeting these pathways reportedly attenuate renal fibrosis and improve renal functional indices (12,14-16). Moreover, 5/6Nx mice exhibit lipid-associated stress responses, characterized by renal fatty-acid compositional shifts that are accompanied by tubular ER-stress and inflammatory signaling and are linked to fibrotic responses (17). This model has been applied for the development and elucidation of the mechanism of action of drugs that protect renal function, such as empagliflozin (18) and sesamol (19), as well as those that improve uremia, including AST-120 (11). Collectively, these features make 5/6Nx mice a suitable model to evaluate candidate interventions targeting CKD-relevant functional and structural outcomes.

Fatty acids play crucial roles in renal metabolism and function in CKD pathogenesis. Renal epithelial cells primarily rely on fatty acids for energy, whereas CKD leads to lipid accumulation in the tubular epithelium and decreased fatty acid oxidation, contributing to fibrosis and disease progression (20). Mitochondrial dysfunction and defects in fatty acid oxidation are universally implicated in acute kidney injury (AKI) and CKD (21). Medium-chain fatty acid metabolism has emerged as a determinant of renal fibrogenesis, as impaired medium-chain fatty acid oxidation in fibrotic kidneys is linked to abnormal mitochondrial homeostasis and worsened fibrosis (22). Notably, pentadecanoic acid (C15:0), a major odd-chain fatty acid, has been reported to restore mitochondrial function in human cell-based assays, although its renal relevance *in vivo* remains to be established (23). These findings raise interest in lipid-derived candidates as potential adjunctive interventions in CKD.

In recent years, oils rich in odd-numbered fatty acids derived from *Aurantiochytrium*, a microalga belonging to the Stramenopiles group, have gained recognition worldwide as valuable components of nutraceuticals and cosmetics. For example, the ethanol extract of *Aurantiochytrium* improves spatial learning and memory in Senescence-Accelerated Mouse-Prone 8 (SAMP8) mice and increases neurogenesis in the hippocampus of this animal model in age-associated diseases (24). Furthermore, the ethanolic extract of *Aurantiochytrium* downregulates lipopolysaccharide (LPS)-induced pro-inflammatory cytokine induction including interleukin (IL)-1 β , IL-6, and tumor necrosis factor (TNF)- α in murine macrophage RAW264 cells (25). The ethanolic extract of *Aurantiochytrium* also exhibits antidepressant-like effects through reduced expression of pro-inflammatory-related genes in the limbic area of male ICR mice (24). Pentadecyl[®] (Table I) is a triglyceride whose fatty acid composition consists mainly of pentadecanoic acid (C15:0), and is extracted from *Aurantiochytrium limacinum* (26). Notably, Pentadecyl[®] has shown therapeutic effects against insulin secretion and glucose intolerance by downregulating the expression of ER stress-related

genes in the pancreatic islets of mice with Asian type 2 diabetes (26). More recently, our research group has shown that Pentadecyl[®] selectively suppresses LPS-evoked IL-6 and IL-1 β transcription by attenuating STAT3 phosphorylation in BV-2 microglial cells (27). Notably, indoxyl sulfate, a uremic solute accumulated in 5/6Nx mice (11), has been reported to promote tubulointerstitial injury via STAT3 activation in subtotally nephrectomized rats (28) and to suppress cell proliferation via ER stress in human proximal tubular cells (29). These observations suggest that Pentadecyl[®] may modulate CKD-relevant pathways involving STAT3 activation and ER stress. Moreover, a human cell-based study demonstrated that pentadecanoic acid (C15:0), a major constituent of Pentadecyl[®], restores mitochondrial function, a key contributor to CKD progression (23). However, prior studies on *Aurantiochytrium*-derived extracts, Pentadecyl[®], and its major constituent pentadecanoic acid (C15:0) have been limited to non-renal contexts. Therefore, it remains unclear whether these activities translate into improvements in CKD-relevant renal outcomes.

In current clinical practice, guideline-directed pharmacotherapy for CKD is based on renin-angiotensin system inhibitors and sodium-glucose cotransporter-2 inhibitors to slow disease progression (30). However, substantial residual risk of kidney function decline persists, and treatment optimization may be constrained by tolerability (31,32), supporting continued interest in adjunctive strategies that are orally feasible and compatible with standard therapy. Nutrients and natural products are valuable resources for developing such adjunctive interventions (33). Pentadecanoic acid (C15:0) supplementation has been reported to be well tolerated in a randomized controlled trial (34), supporting the preclinical evaluation of Pentadecyl[®] as a potential nutritional adjunct rather than a replacement for guideline-directed therapy. Clinically, CKD is evaluated and staged primarily based on glomerular filtration rate (GFR), commonly estimated from serum CRE level, and preservation of kidney function is a central therapeutic goal (35,36). As chronic structural lesions such as tubulointerstitial fibrosis (37) and interstitial fibrosis and tubular atrophy (38), together with glomerulosclerosis (39), are linked to subsequent loss of kidney function, assessing serum CRE together with BUN provides clinically relevant functional endpoints for evaluating candidate interventions.

In the present study, we aimed to examine whether Pentadecyl[®] administration is associated with changes in renal functional and structural indices relevant to CKD in the 5/6Nx mouse model. We hypothesized that Pentadecyl[®] administration can attenuate renal functional deterioration, consistent with its reported modulatory effects on STAT3-dependent inflammatory signaling (27) and ER stress (26), and with mitochondrial effects of its major fatty acid component, pentadecanoic acid (C15:0) (23), in a 5/6Nx mouse model that recapitulates uremic solute accumulation (11) and CKD-relevant tubular stress pathways (17). To test this hypothesis, we administered Pentadecyl[®] to 5/6Nx mice and evaluated survival and body weight, serum biochemistry including CRE and BUN levels as renal function-related indices, proteins, electrolytes/minerals, and metabolic markers, and histological indices of renal injury.

Table I. Fatty acid composition of Pentadecyl®.

Compositional formula	Fatty acid composition	Content (%)
C ₄₅ H ₈₆ O ₆	Pentadecanoic acid (C15:0)	2.1
	Tetradecanoic acid (C14:0)	
	Tridecanoic acid (C13:0)	
C ₄₆ H ₈₈ O ₆	Pentadecanoic acid (C15:0)	8.0
	Pentadecanoic acid (C15:0)	
	Tridecanoic acid (C13:0)	
C ₄₆ H ₈₈ O ₆	Pentadecanoic acid (C15:0)	8.0
	Tetradecanoic acid (C14:0)	
	Tetradecanoic acid (C14:0)	
C ₄₇ H ₉₀ O ₆	Pentadecanoic acid (C15:0)	18.8
	Pentadecanoic acid (C15:0)	
	Tetradecanoic acid (C14:0)	
C ₄₈ H ₉₂ O ₆	Pentadecanoic acid (C15:0)	30.6
	Pentadecanoic acid (C15:0)	
	Pentadecanoic acid (C15:0)	
C ₄₉ H ₉₄ O ₆	Pentadecanoic acid (C15:0)	22.3
	Pentadecanoic acid (C15:0)	
	Hexadecanoic acid (C16:0)	
C ₅₀ H ₉₆ O ₆	Pentadecanoic acid (C15:0)	11.7
	Pentadecanoic acid (C15:0)	
	Heptadecanoic acid (C17:0)	
C ₅₀ H ₉₆ O ₆	Pentadecanoic acid (C15:0)	11.7
	Hexadecanoic acid (C16:0)	
	Hexadecanoic acid (C16:0)	
C ₅₁ H ₉₈ O ₆	Pentadecanoic acid (C15:0)	4.5
	Hexadecanoic acid (C16:0)	
	Heptadecanoic acid (C17:0)	
C ₅₂ H ₁₀₀ O ₆	Pentadecanoic acid (C15:0)	2.0
	Heptadecanoic acid (C17:0)	
	Heptadecanoic acid (C17:0)	
C ₄₄ H ₈₄ O ₆	Tridecanoic acid (C13:0)	2.0
	Tridecanoic acid (C13:0)	
	Pentadecanoic acid (C15:0)	

Materials and methods

Extraction and purification of Pentadecyl® from Aurantiochytrium oil. Pentadecyl® was extracted and purified from *Aurantiochytrium limacinum* at Sea Act Co. Ltd. (Tokyo, Japan) following a previously described method (26). Briefly, the collected *Aurantiochytrium limacinum* cells were washed with water, and hexane was added to extract the fat. The hexane solution was then cooled and reprocessed to obtain white Pentadecyl® crystals.

5/6-Nephrectomy and Pentadecyl® treatments. Adult male C57BL/6J mice were purchased from Sankyo Laboratory Service Co. Ltd. (Tokyo, Japan) and maintained on a 12-h light/dark cycle at 24±1°C with *ad libitum* access to food and water. All efforts were made to minimize animal anguish and

reduce the number of animals used, in accordance with the international guidelines for the care and welfare of animals. All animal experiments were conducted in accordance with the guidelines approved by the Nihon University Animal Care and Use Committee (Tokyo, Japan; experiment numbers AP24PHA010-1, AP19PHA021-1, and AP19PHA021-2). Eight-week-old male C57BL/6 mice were subjected to 5/6Nx to induce renal dysfunction as described previously (40). This operation was conducted in two surgical stages: first, resection of the upper and lower poles of the left kidney, and right kidney resection 1 week after the first surgery. Control mice underwent a sham operation in which the kidney was exposed and the abdominal wall was then closed with sutures. Mice were anesthetized with isoflurane (induction: 4%; maintenance: 2.0% in oxygen) delivered via a precision vaporizer during surgical procedures. Depth of anesthesia was confirmed by the absence of pedal withdrawal and corneal reflexes. Surgical procedures were performed at Sankyo Lab Service (Tokyo, Japan). The allocation procedure used by the vendor for sham vs. 5/6Nx assignment was not accessible to the investigators. The following week after the last surgery, the mice were divided into the following groups: i) sham operated (control) mice with drinking water containing 2.0% ethanol (vehicle) (Cont-V, n=10); ii) 5/6Nx mice with drinking water containing 2.0% ethanol (CKD-V, n=7); and iii) 5/6Nx mice treated with drinking water containing 0.2 g/l Pentadecyl® (CKD-P, n=7). 5/6Nx mice were assigned to vehicle or Pentadecyl® group based on institutional ID order, with the first half allocated to the vehicle group and the second half allocated to the Pentadecyl® group. Pentadecyl® was dissolved in ethanol (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan), as previously described (26), diluted with drinking water to the above concentration, and administered for 8 weeks. The concentration of 0.2 g/l was chosen empirically to allow continuous oral exposure via drinking water over the 8-week period under conditions maintaining *ad libitum*, because no established pharmacological dose of Pentadecyl® is available for 5/6Nx CKD models. All mice were weighed twice a week, and their daily body weight gain was calculated. Water and food consumption were measured weekly, and the average daily water and food intake was calculated. As dosing via drinking water can vary among animals, we estimated Pentadecyl® intake (mg/kg/day) from measured water consumption and contemporaneous body weight.

Measurement of serum biochemical parameters. After 8 weeks of drug administration, blood was collected from the hearts of deeply anesthetized mice, as previously described (40). Mice were anesthetized with isoflurane (4% in oxygen) delivered via a precision vaporizer, and depth of anesthesia was confirmed by the absence of pedal withdrawal and corneal reflexes. After cardiac puncture, exsanguination served as a secondary physical method to ensure death. Death was confirmed by cessation of respiration, absence of heartbeat, and loss of reflexes prior to tissue collection. Mice were fasted and deprived of water for 4 h prior to blood collection. The blood was centrifuged at 1,500 rpm for 15 min in Eppendorf 5417R centrifuge (Eppendorf AG, Hamburg, Germany) at room temperature, and the resulting supernatant was used as the serum sample. Serum biochemical parameters, viz. albumin (ALB), BUN,

Table II. Effects of Pentadecyl® on serum biochemical parameters in 5/6Nx mice.

Parameters	Cont-V (n=10)	CKD-V (n=7)	CKD-P (n=7)
TP, g/dl	4.59±0.26	4.45±0.20	4.61±0.20
ALB, g/dl	2.88±0.14	2.87±0.15	2.93±0.10
BUN, mg/dl	31.37±2.91	77.23±5.29 ^a	69.33±6.01 ^{a,b}
CRE, mg/dl	0.12±0.02	0.32±0.07 ^a	0.25±0.02 ^{a,c}
UA, mg/dl	2.94±0.94	2.87±0.54	3.27±1.42
Na, mEq/l	154.60±2.07	153.90±7.15	153.00±2.89
K, mEq/l	6.09±0.46	6.23±0.81	7.14±4.14
Cl, mEq/l	109.00±2.63	106.7±4.72	107.00±1.92
Ca, mg/dl	9.30±0.19	10.04±0.74 ^d	10.59±0.60 ^e
IP, mg/dl	8.24±0.71	7.53±0.33	8.87±2.47
Mg, mg/dl	3.85±0.22	4.46±0.56 ^d	4.26±0.52
T-CHO, mg/dl	76.90±10.95	87.71±9.74	89.29±5.28 ^d
GLU, mg/dl	233.70±31.49	282.40±29.14 ^d	256.10±45.82
GA, %	3.40±0.44	3.99±0.61	3.93±0.61

^aP<0.05, ^bP<0.001 and ^cP<0.0001 vs. sham-operated mice. ^dP<0.01 and ^eP<0.05 vs. vehicle-treated 5/6Nx mice. Data are presented as mean ± SD. Statistical differences were analyzed using one-way ANOVA with Tukey's post-hoc test (n=7-10). TP, Total protein; ALB, albumin; BUN, blood urea nitrogen; CRE, creatinine; UA, uric acid; Na, sodium; K, potassium; Cl, chloride; Ca, calcium; Mg, magnesium; IP, inorganic phosphorus; T-CHO, total cholesterol; GLU, glucose; GA, glycoalbumin.

calcium (Ca), CRE, glucose (GLU), glycoalbumin (GA), inorganic phosphorus (IP), potassium (K), magnesium (Mg), sodium (Na), total cholesterol (T-CHO), total protein (TP), and uric acid (UA) were measured at the Nagahama Institute for Biochemical Science, Oriental Yeast Co., Ltd. (Japan). Following blood sample collection, the kidney tissue was rapidly dissected from each animal and infiltrated into a 10% formalin solution (FUJIFILM Wako Pure Chemical Corporation).

Histological assessment of kidneys. Post-fixed kidneys were embedded in paraffin and sectioned on a microtome to a thickness of 4 µm, as previously described (41). For the histological assessment of kidney damage, paraffin-embedded sections were stained with hematoxylin and eosin (H&E; Sakura Finetek Japan Co., Ltd. Tokyo, Japan). Images were visualized using a BZ-X810 optical microscope (KEYENCE, Osaka, Japan) at 20x magnification and analyzed using the BZ-X810 analysis application BZ-H4A (KEYENCE). Each sample was evaluated using five fields of view. Renal morphology was evaluated via quantitative morphometric measurement of glomerular and tubular cross-sectional areas on H&E-stained sections. Glomerular area was delineated by tracing the boundary of intact glomerular profiles; tangentially sectioned or incomplete profiles were excluded. For each mouse, the glomerular area within 7 to 29 glomeruli was measured and averaged to obtain a representative value. Tubular area was quantified in five non-overlapping cortical fields per section, excluding fields containing obvious artifacts. No thresholds were applied, and all measurements were treated as continuous variables. To visualize dispersion within groups, frequency distributions were generated using individual glomerular and tubular area measurements; the results are presented as relative

frequency, normalized to the total number of measurements in each group. Slides and images were coded, and observers were blinded to both surgical group and treatment during outcome assessment.

Statistics. Statistical analyses were performed using GraphPad Prism 9 software (GraphPad Software Inc., San Diego, CA, USA). All data are expressed as mean ± standard deviation (SD) or standard error of the mean (SE). Unpaired Student's t-test was used for comparisons between two groups. One-way analysis of variance (ANOVA) with Tukey's post hoc test was used for Figs. 3, 4, and Table II, or two-way ANOVA with Tukey's post hoc test was used for Fig. 2. Survival data were analyzed using the Kaplan-Meier method with a log-rank test. Statistical significance was set at P<0.05. A formal a priori sample size calculation was not performed because reliable effect-size estimates for Pentadecyl® in the 5/6Nx model were unavailable at the planning stage. Group sizes were determined based on feasibility and animal-welfare considerations and are comparable to those used in prior 5/6Nx mouse studies (e.g., n ≤10 per group) (42-44).

Results

Pentadecyl® does not influence survival duration in 5/6Nx mice. First, we examined the effect of Pentadecyl® on the survival of mice that had undergone 5/6Nx. Treatment with Pentadecyl® or vehicle (2.0% ethanol) was initiated in 10-week-old mice (1 week after the last operation). With a cut-off set at 18 weeks (126 days of age), no mortality was observed in the Cont-V group during the 8-week treatment period (Fig. 1A). As shown in Fig. 1A, the median survival duration of CKD-V and CKD-P mice was 126 days, and no significant difference was

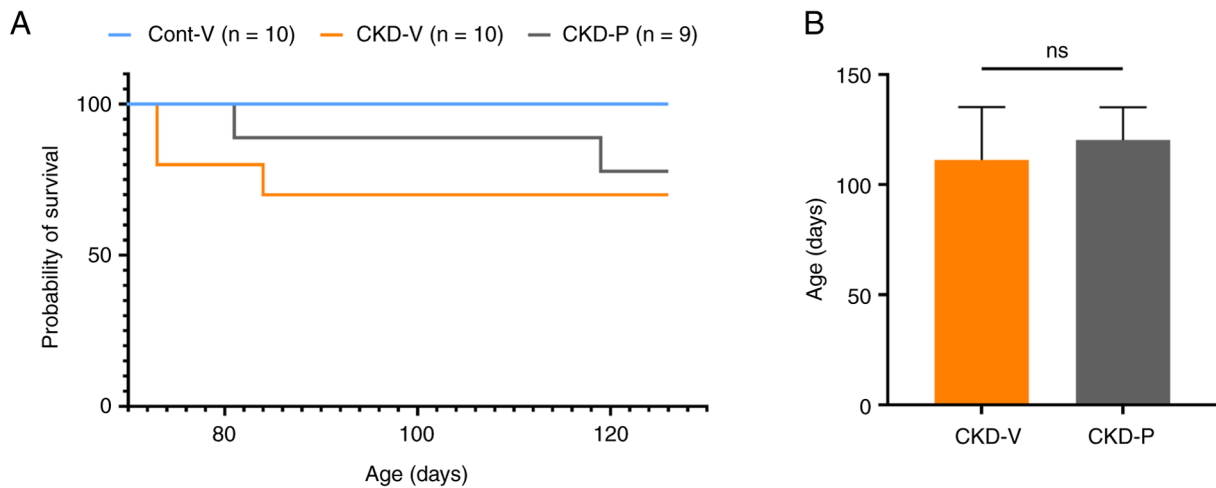


Figure 1. Effect of Pentadecyl® on the survival of 5/6Nx mice. (A) Survival curves of sham-operated mice treated with vehicle and 5/6Nx mice treated with vehicle or Pentadecyl®. Survival curves were analyzed using the Kaplan-Meier method with the log-rank test ($n=9-10$; $P=0.1971$). (B) Maximum lifespan of 5/6Nx mice treated with vehicle or Pentadecyl®. Data are presented as mean $\pm$ SD, with a cut-off set at 18 weeks (126 days of age) ($n=7-10$; $P=0.3456$). ns, not significant; Cont-V, Sham-operated mice treated with vehicle; CKD-V, 5/6Nx mice treated with vehicle; CKD-P, 5/6Nx mice treated with Pentadecyl®.

observed between these groups. Thus, only two individuals died in each group. Moreover, the average survival duration of CKD-P mice was 120.2 days, showing no significant difference compared with that of CKD-V mice (111.2 days; Fig. 1B). These results indicate that Pentadecyl® treatment does not have a lifespan-prolonging effect in 5/6Nx mice.

Pentadecyl® improves body weight gain without affecting food or water intake in 5/6Nx mice. The effect of Pentadecyl® on body weight gain in 5/6Nx mice was investigated. While the CKD-V group exhibited a reduced increase in body weight compared to the Cont-V group, Pentadecyl® administration significantly improved body weight gain in mice with CKD (Fig. 2A). In contrast, no significant differences in the daily food intake were observed among the three groups throughout the experimental period (Fig. 2B). Additionally, both the CKD-V and CKD-P groups showed a sustained increase in water intake compared with the Cont-V group; however, no significant differences were observed between the CKD-V and CKD-P groups (Fig. 2C). Estimated Pentadecyl® exposure ranged from 61.17 to 110.59 mg/kg/day (overall mean $\pm$ SD, 76.71 ± 10.68 mg/kg/day) in CKD-P mice with complete longitudinal records across 1-8 weeks. No significant differences in serum parameters were observed between vehicle-treated and water-treated sham-operated mice (data not shown).

Pentadecyl® improves serum parameters indicative of renal function in 5/6Nx mice. We evaluated the effects of Pentadecyl® administration on various serum parameters in 5/6Nx mice. As shown in Table II, compared with the Cont-V group, the CKD-V group exhibited significant increases in serum levels of BUN, CRE, Ca, Mg, and GLU. Serum Ca levels in the CKD-P group remained elevated, similar to those in the CKD-V group, whereas BUN and serum CRE levels in the CKD-P group were significantly lower than those in the CKD-V group (Table II). However, BUN and serum CRE levels in the CKD-P group were not restored to Cont-V levels and remained significantly elevated compared with those in the

Cont-V group (Table II). In contrast, serum T-CHO levels were significantly elevated only in the CKD-P group compared to those in the Cont-V group. No notable changes were observed in serum TP, ALB, UA, Na, K, Cl, IP, or GA levels among the three groups (Table II).

Effect of Pentadecyl® on renal histological changes in 5/6Nx mice. We assessed the influence of Pentadecyl® on histopathological changes in the kidneys of 5/6Nx mice through hematoxylin and eosin (H&E) staining (Fig. 3A). Compared with the glomerular area in the Cont-V group, both the CKD-V and CKD-P groups showed significant increases. However, no significant difference was observed in glomerular area between the CKD-V and CKD-P groups ($P=0.8555$; Fig. 3B). To investigate the distribution of glomerular area, a relative frequency comparison was performed across the three groups (Fig. 3C). In the Cont-V group, the majority of glomeruli were distributed in the range of $2,001-2,500 \mu\text{m}^2$, whereas the majority in both the CKD-V and CKD-P groups were in the range of $2,501-3,000 \mu\text{m}^2$. The histogram for the CKD-P group appeared to show a lower proportion of glomeruli in the $3,001-3,500 \mu\text{m}^2$ range and a higher proportion in the $2,501-3,000 \mu\text{m}^2$ range than those for the CKD-V group; the histograms are presented for descriptive purposes and were not subjected to statistical testing. Similar to the determination of glomerular histopathological changes, we evaluated the effect of Pentadecyl® on renal tubular histopathological changes in 5/6Nx mice using H&E staining (Fig. 4). Both the CKD-V and CKD-P groups showed a significant increase in renal tubular area compared to the Cont-V group. However, no significant differences were observed between the CKD-V and CKD-P groups ($p=0.6796$; Fig. 4B). A comparison of the relative frequency of renal tubular areas across the groups revealed that most tubules in the Cont-V group were distributed within the range of $156-235 \mu\text{m}^2$, whereas the majority in both the CKD-V and CKD-P groups were within the range of $236-395 \mu\text{m}^2$ (Fig. 4C). Tubules in the range of $2,636-16,033 \mu\text{m}^2$ appeared less frequent in the CKD-P group than in the CKD-V group

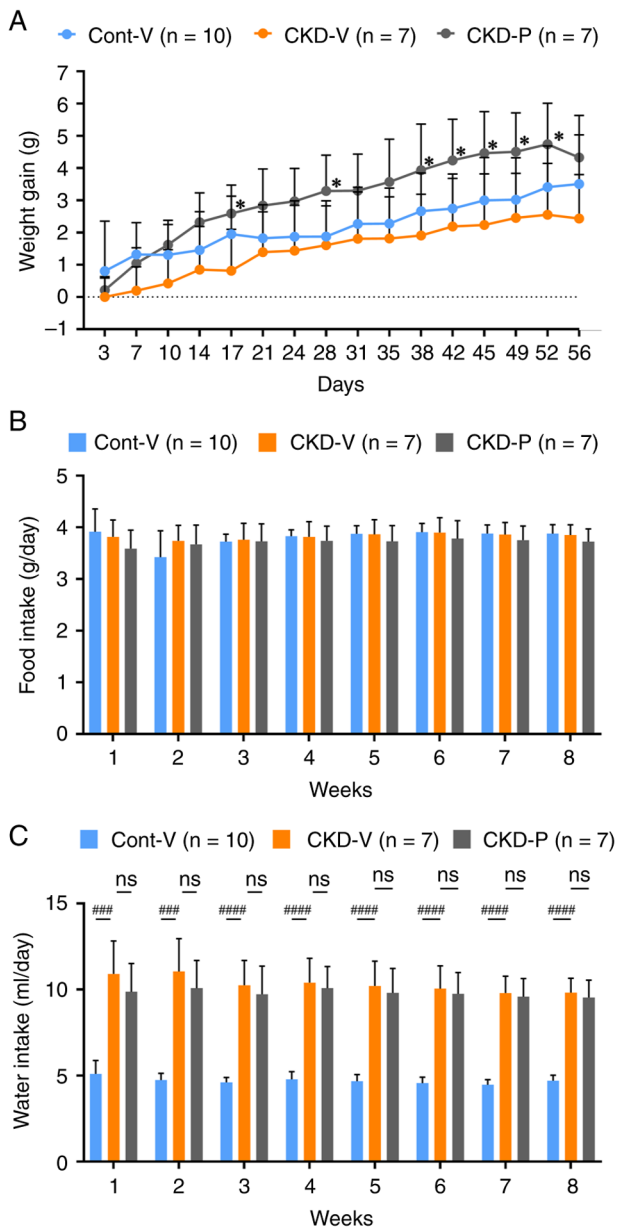


Figure 2. Change in body weight gain, food intake and water intake. Changes over time in (A) cumulative body weight gain, (B) food intake and (C) water intake in 5/6Nx mice treated with either vehicle or Pentadecyl®, as well as in sham-operated mice treated with the vehicle. Data are presented as mean $\pm$ SD. Statistical differences were determined using two-way ANOVA with Tukey's post-hoc test ($n=7-10$). * $P<0.05$ vs. CKD-V mice. *** $P<0.001$ and **** $P<0.0001$. ns, not significant; Cont-V, Sham-operated mice treated with vehicle; CKD-V, 5/6Nx mice treated with vehicle; CKD-P, 5/6Nx mice treated with Pentadecyl®.

(Fig. 4C); the histograms are presented for descriptive purposes and were not subjected to statistical testing.

Pentadecyl® treatment was associated with lower BUN and serum CRE levels but was not associated with significant differences in mean glomerular area, mean tubular area, or survival during the 8-week period.

Discussion

CKD is characterized by progressive loss of kidney function and structural remodeling. Despite guideline-directed

therapies, a residual risk of progressive kidney function decline persists in CKD, supporting continued interest in adjunctive strategies that are compatible with standard care. To the best of our knowledge, this is the first study to demonstrate that administration of Pentadecyl®, a functional triglyceride derived from *A. limacinum*, to 5/6Nx mice suppresses the elevation in BUN and CRE levels, indicating partial improvement in renal function, whereas quantitative histological indices showed limited changes.

Chronic oral administration of Pentadecyl® significantly improved body weight gain in 5/6Nx mice compared to that in the vehicle-treated group, despite no changes being observed in food or water intake, or survival duration. This finding could be consistent with attenuation of CKD-associated metabolic status, such as cachexia-like weight loss, which is frequently observed in patients with advanced renal dysfunction. However, we did not perform direct assessments of energy expenditure, body composition, or muscle and fat mass. CKD-induced metabolic abnormalities, including chronic inflammation, insulin resistance, and impaired protein metabolism contribute to muscle wasting and weight loss (45,46). Therefore, an alternative interpretation is that Pentadecyl® may have attenuated 5/6Nx-associated muscle wasting or proteolysis (47), which cannot be distinguished from other mechanisms based on the current dataset. Future studies incorporating the assessment of muscle atrophy and muscle catabolic markers, such as Atrogin-1 and MuRF1, are needed to clarify the basis of the body weight difference. In contrast, while water intake significantly increased in vehicle-treated 5/6Nx mice, Pentadecyl® did not alter this increase. Water intake is closely associated with kidney filtration function in patients with CKD (48). Human clinical studies have indicated a protective effect of high water intake on kidney function (49). Moreover, increased water intake slows the progression of chronic renal failure in 5/6Nx rats (50). The increased water intake observed in the treated group compared to that in the sham-operated group suggests that Pentadecyl® does not inhibit the potential protective effect of increased fluid intake on kidney function in mice with CKD. These findings imply that Pentadecyl® treatment does not interfere with this beneficial physiological adaptation. To this end, compared to conventional pharmacological treatments, natural lipid-derived products such as Pentadecyl® may have a superior safety profile.

In general, CKD mouse models, including 5/6Nx mice, have been reported to exhibit elevated BUN and serum CRE levels, reflecting renal dysfunction (40,51). Additionally, CKD models often display alterations in electrolyte balance, such as changes in K, Na, Ca, and phosphate levels (51). Consistent with these findings, the present study demonstrated that BUN, serum CRE, Ca, and Mg levels increased in the CKD-V group, suggesting impaired renal function in 5/6Nx mice. Oral administration of Pentadecyl® in drinking water significantly suppressed the elevation of BUN and serum CRE levels in the animals. However, Pentadecyl® did not prevent an increase in electrolyte imbalance, including serum Ca and Mg levels, in 5/6Nx mice. Notably, Pentadecyl® ameliorates insulin secretion and glucose tolerance in Asian mice with type 2 diabetes (26). In the current study, serum GLU levels tended to decrease in Pentadecyl®-treated mice with CKD, which may reflect the indirect metabolic benefits of improved renal function.

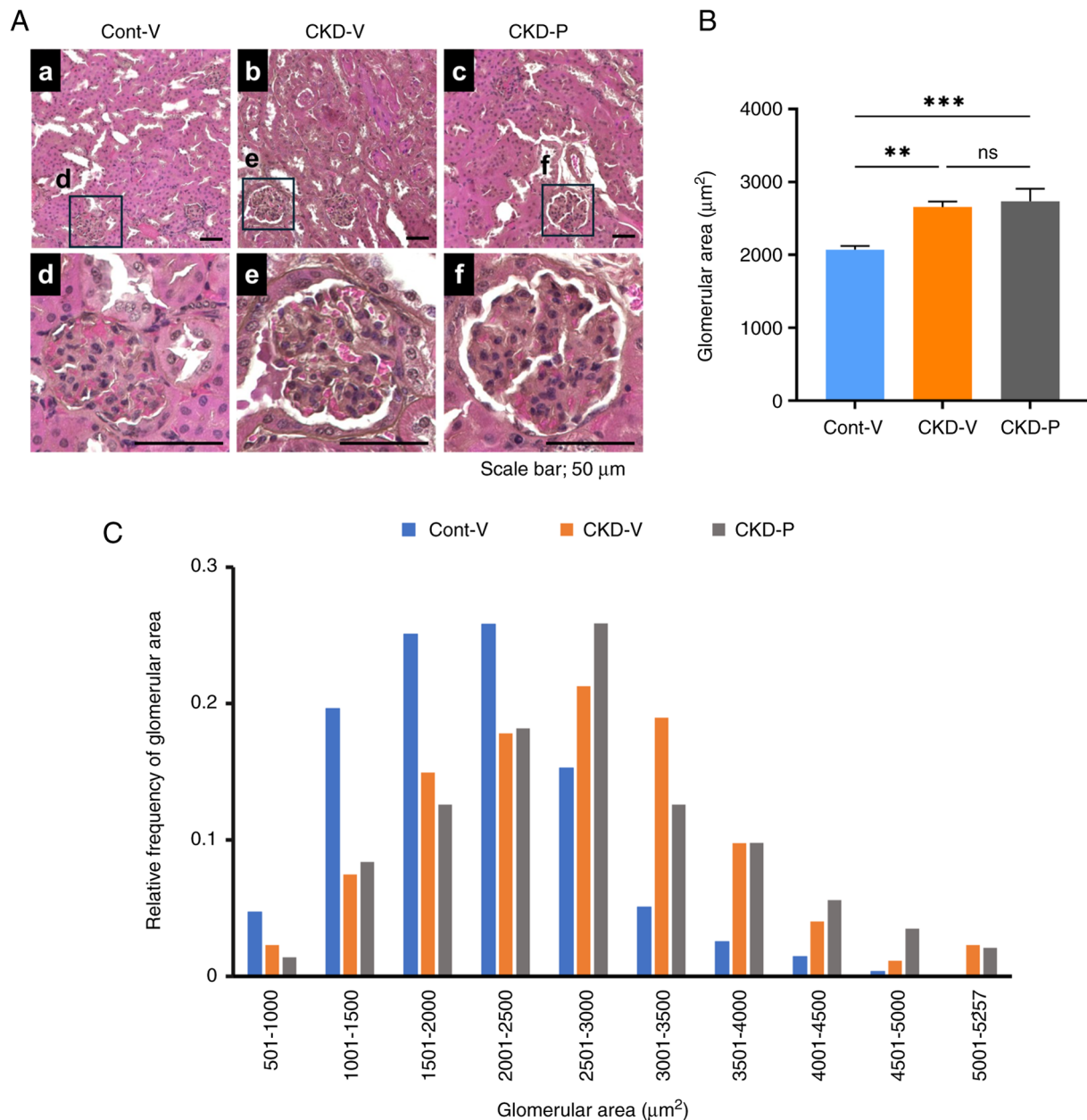


Figure 3. Effects of Pentadecyl® on glomerular histopathological changes and the increase in glomerular surface area in 5/6Nx mice. (A) Representative histological photomicrograph of glomeruli by H&E staining in the three groups (sham mice, vehicle-treated 5/6Nx mice, Pentadecyl®-treated 5/6Nx mice) after 8 weeks of administering drinking water. Panels (a-c) are low-magnification views, and panels (d-f) are higher-magnification views corresponding to the boxed glomeruli in panels (a-c), respectively. Scale bars, 50 µm. (B) Average glomerular area (17-36 glomeruli per sample) measured for each group, expressed as mean ± SE. Statistical difference was analyzed using one-way ANOVA with Tukey's post-hoc test (n=7-10). **P<0.01 and ***P<0.001; n=7-10. (C) Relative frequency distribution of glomerular area in the three groups. ns, not significant; Cont-V, Sham-operated mice treated with vehicle; CKD-V, 5/6Nx mice treated with vehicle; CKD-P, 5/6Nx mice treated with Pentadecyl®.

Although treatment with Pentadecyl® resulted in a slight but significant increase in serum T-CHO levels, these values remained within the normal physiological range for C57BL/6J mice (1.8-3.9 mmol/l; approximately 70-151 mg/dl) (52). Nevertheless, this increase should be acknowledged as a potential lipid-related off-target effect that warrants monitoring in future studies. This is particularly relevant because CKD is associated with characteristic lipoprotein abnormalities and elevated cardiovascular risk, and conventional cholesterol measures may not fully capture qualitative atherogenic changes in lipoproteins (53). Accordingly, subsequent studies should incorporate a more detailed lipid assessment,

including LDL/HDL fractions, triglycerides, and non-HDL cholesterol, together with relevant metabolic endpoints, to better characterize the lipid effects of Pentadecyl® under CKD conditions. These results suggest that Pentadecyl® partially alleviates renal dysfunction and may help prevent complications in a CKD mouse model. Moreover, to confirm the changes in renal characteristics of 5/6Nx mice, we evaluated the changes in renal glomerular hypertrophy and tubular dilatation, which are typical histological markers of kidney dysfunction in CKD (9,54). Previous studies using animal models of CKD have reported increases in renal glomerular and tubular areas compared to those in the control group (55). In agreement

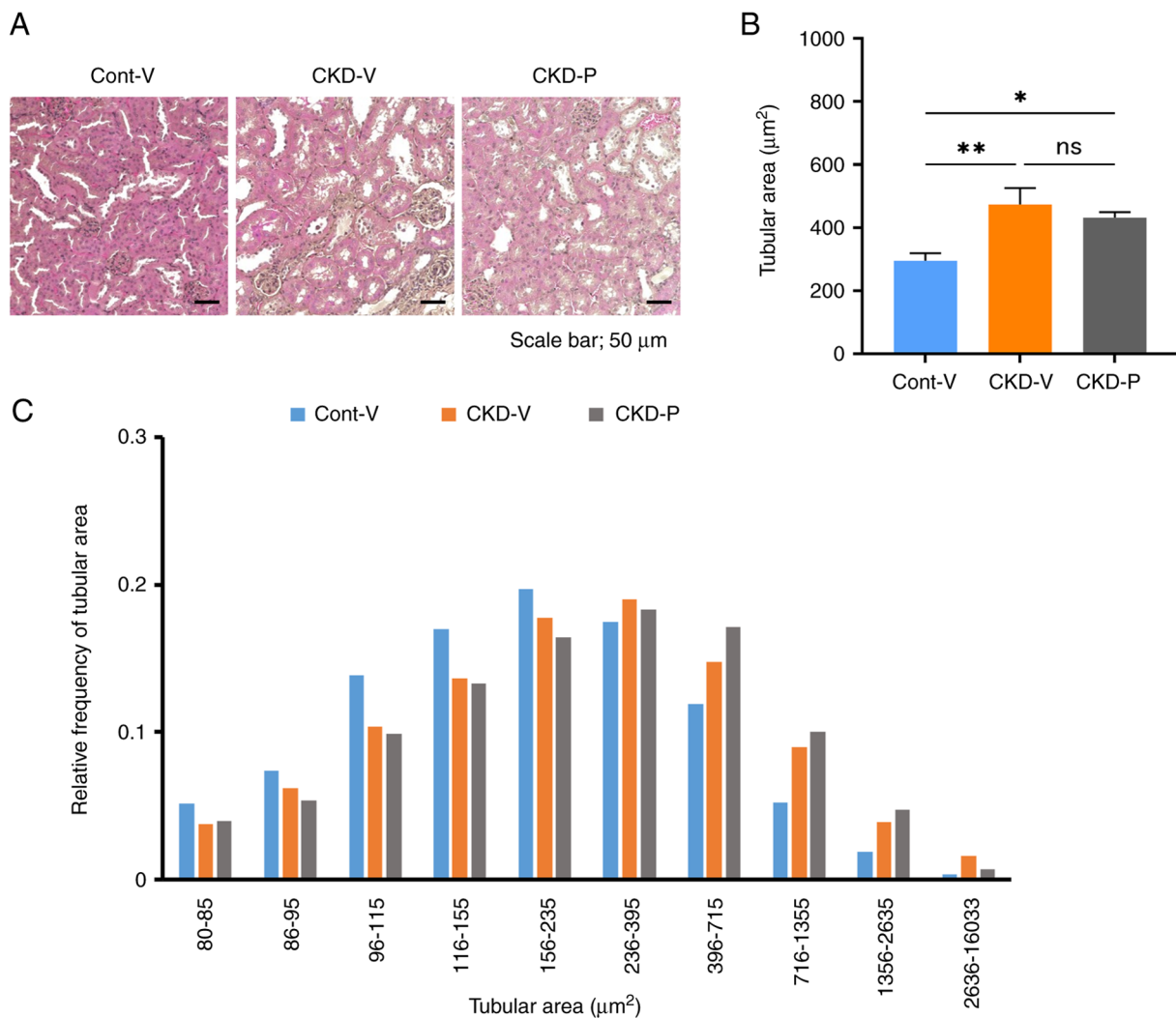


Figure 4. Effects of Pentadecyl[®] on renal tubular histopathological changes and tubular dilation in 5/6Nx mice. (A) Representative histological photomicrograph of renal tubules by H&E staining in the three groups (sham mice, vehicle-treated 5/6Nx mice, Pentadecyl[®]-treated 5/6Nx mice) after 8 weeks of administering drinking water. Scale bars, 50 μm. (B) Tubular area, defined as the lumen excluding vascular and glomerular spaces. The areas of 150–693 tubules per sample were measured, and the data are expressed as mean ± SE. Statistical differences were determined using one-way ANOVA with Tukey's post-hoc test (n=7–10). *P<0.05 and **P<0.01. (C) Relative frequency distribution of tubular area in the three groups. ns, not significant; Cont-V, Sham-operated mice treated with vehicle; CKD-V, 5/6Nx mice treated with vehicle; CKD-P, 5/6Nx mice treated with Pentadecyl[®].

with these findings, in the present study, glomerular hypertrophy and tubular dilatation were significantly increased in vehicle-treated 5/6Nx mice compared to those in control mice. Importantly, Pentadecyl[®] treatment did not significantly improve these histological changes despite improvements in BUN and serum CRE levels. BUN and serum CRE levels can be influenced by factors beyond glomerular filtration, including protein catabolism and muscle mass (56), which is particularly relevant when body weight differs across groups. Therefore, future studies should incorporate muscle-independent filtration markers, including serum cystatin C (57) and symmetric dimethylarginine (58,59), and direct assessment of GFR using clearance-based methods such as fluorescein isothiocyanate-sinistrin. In addition, modification of chronic structural lesions may require a longer treatment duration than that used in the present study, and the present histological assessment relied largely on size-based morphometry, which may be insufficient to capture treatment effects on fibrotic remodeling or tubular injury. Therefore, future studies should

evaluate longer dosing periods and include additional structural endpoints that more directly capture chronic remodeling, such as fibrosis quantification using Sirius Red or Masson's trichrome staining and immunostaining for collagen and α-smooth muscle actin.

The fatty acid composition of Pentadecyl[®] primarily consists of pentadecanoic acid (C15:0), which accounts for more than 60% of the total content, followed by tridecanoic acid (C13:0), hexadecanoic acid (C16:0), and heptadecanoic acid (C17:0) (60). Additionally, Pentadecyl[®] selectively inhibits the LPS-induced induction of IL-6 and IL-1β, but not TNF-α, in BV-2 murine microglial cells (27). At non-cytotoxic concentrations, pentadecanoic acid (C15:0) suppresses LPS-induced pro-inflammatory cytokines, including IL-6, IL-1β, and TNF-α, in both human intestinal Caco-2 cells and the MODE-K murine enterocyte cell line (61). Similarly, hexadecanoic acid (C16:0) and heptadecanoic acid (C17:0) attenuate the production of these inflammatory cytokines in LPS-treated BV-2 cells (62). Pentadecanoic acid has been

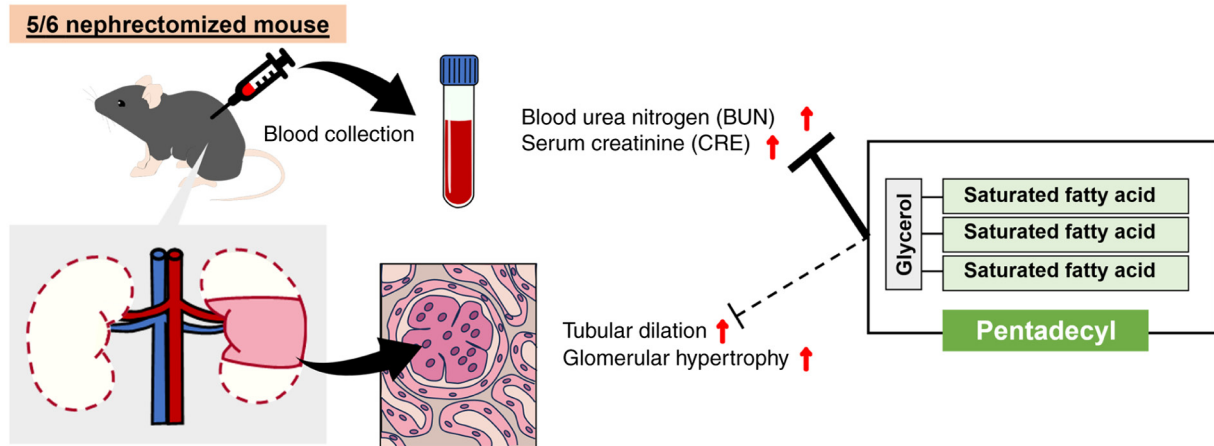


Figure 5. Schematic illustration of renoprotective effect of Pentadecyl® in the 5/6 nephrectomized mouse model of chronic kidney disease.

reported to have anti-inflammatory and anti-fibrotic effects in *in vitro* and *in vivo* studies, albeit in pathological models of liver injury other than CKD (23,63). Although the exact molecular pathways underlying the effects of Pentadecyl® on renal outcomes and the key active constituent(s) remain to be elucidated, the known anti-inflammatory and metabolic activities of Pentadecyl® are postulated to be extensively involved. Further studies are needed to directly assess renal inflammatory and fibrotic pathways and to clarify the mechanisms underlying the observed changes in renal functional indices.

This study has some limitations that should be addressed in future studies. First, the background genetic strain of mice has been reported to influence the pathophysiology of renal dysfunction in 5/6Nx models. C57BL/6 mice, similar to those used in this study, do not recapitulate some of the symptoms of CKD in humans, including cardiac fibrosis, proteinuria, glomerulosclerosis, and hypertension (64,65). Therefore, the renal protective effect of Pentadecyl® may need to be re-evaluated in 5/6Nx mice using strains other than C57BL/6 as well as other models of renal dysfunction, such as the adenine diet (8), oxalate diet (66), and aristolochic acid nephropathy (67) models. Second, similar to this study, most preclinical studies of CKD have been conducted in male animals (19). However, female C57BL/6 mice exhibit a similar decline in GFR compared to male mice but do not show the same changes in BUN, serum Ca levels, or muscle atrophy (47). In addition, the prevalence of CKD is higher in postmenopausal women (68). These findings suggest that future studies should investigate the protective effects of these interventions in female mice, including those in the postmenopausal state. Third, Pentadecyl® was administered via drinking water, which may introduce inter-individual variability in exposure due to differences in water consumption. Because only a single concentration of Pentadecyl® was examined, which was empirically selected due to the lack of an established dose for 5/6Nx models, dose-response relationships were not evaluated and the optimal dose range remains unclear. Therefore, we estimated individual intake using measured water intake and contemporaneous body weight; the resulting exposure range was approximately 61.17-110.59 (overall mean $\pm$ SD, 76.71 ± 10.68) mg/kg/day across the treatment period. This range is within the same

order of magnitude as doses reported in mouse studies using *Aurantiochytrium*-derived ethanol extracts administered by oral gavage (e.g., 50-100 mg/kg/day) (24,69). However, as these preparations differ in composition and were delivered by controlled dosing rather than *ad libitum* drinking water, direct comparability is limited. Future studies using controlled dosing, such as oral gavage, would strengthen dose-response interpretation. Fourth, it is necessary to investigate the mechanisms underlying the observed improvements in BUN and serum CRE of Pentadecyl® in animal CKD models. Recently, we demonstrated that Pentadecyl® exerts anti-inflammatory effects by suppressing STAT3 phosphorylation in BV-2 microglial cells (27). Pentadecyl® improves glucose tolerance by reducing the expression of ER stress-related genes in pancreatic islets of mice with type 2 diabetes (26). ER stress and inflammation have been reported to be involved in the transition from AKI to CKD and/or fibrosis (70,71). Such a study on the mechanism of action using *in vivo* models will provide more clinical evidence supporting the therapeutic potential of Pentadecyl® in renal dysfunction, including CKD. Lastly, our histological assessment focused on morphometric area measurements and did not include a standardized semi-quantitative scoring system. Although area-based measurement provides objective, continuous data that are less susceptible to observer-dependent variability associated with scoring systems, future studies incorporating validated histological scoring criteria and larger sample sizes will be necessary to confirm these morphological observations.

In conclusion, the findings of this study provide the first evidence that Pentadecyl® partially attenuates selected functional markers of renal impairment, BUN and serum CRE, in a murine model of CKD (Fig. 5). Notably, the levels of these parameters remained elevated relative to those in the sham group and histological indices showed limited improvement under the present experimental conditions indicating that renal dysfunction was not fully reversed. No overt adverse findings were recorded within the endpoints evaluated; however, the serum T-CHO level increased, indicating that systemic effects cannot be excluded. Further research is warranted to clarify the associated mechanisms of action and optimize treatment strategies for future clinical applications of this promising natural extract.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

KYY curated the data, performed the formal analysis and investigation, and wrote the original draft. HN curated the data, supervised the study, validated the results, and reviewed and edited the manuscript. SF curated the data, performed the formal analysis and investigation, and wrote the original draft. KT performed the formal analysis and investigation and wrote the original draft. KN performed the formal analysis and prepared the figures. TT performed the formal analysis and validated the results. YS conceptualized the study, developed the methodology, provided resources, and reviewed and edited the manuscript. HI developed the methodology, provided resources, and reviewed and edited the manuscript. MT developed the methodology, provided resources, supervised the study, and reviewed and edited the manuscript. HM performed the formal analysis, acquired funding, validated the results, and wrote the original draft. YK conceptualized the study, acquired funding, wrote the original draft, and reviewed and edited the manuscript. HN and YK confirm the authenticity of all the raw data. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

The animal experiments were reviewed and approved by the Nihon University Animal Care and Use Committee (Tokyo, Japan; approval nos. AP24PHA010-1, AP19PHA021-1 and AP19PHA021-2).

Patient consent for publication

Not applicable.

Competing interests

Prof. Yasuhiro Kosuge received research support from Sea Act Co., Ltd. (Tokyo, Japan). The other authors declare that they have no competing interests.

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