

The Influence of Peptides on the Morphofunctional State of Kidneys in Old Rats

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Abstract—More than a quarter of the elderly and senile age population suffers from kidney pathology. For this reason, a prophylaxis of kidney diseases with safe and effective nephroprotectors is a priority of gerontology. This work studies the influence of the polypeptide kidney complex (PKC) and peptides *AED*, *EDL*, and *AEDG*, on the functional state of kidneys in old rats. The administration of the PKC and peptides *AED* and *EDL* increased diuresis by 1.2–1.4 times. The PKC and peptide *AED* reduced the urine protein level and protein excretion by 1.5–2.8 times. The PKC and peptides *AED* and *EDL* increased distal sodium transport by 1.2–1.3 times. The peptides *AED* and *EDL* increased sodium excretion by 1.3 and 1.6 times respectively. The renal effects of the peptide *AEDG* resulted in a 21% reduction of glomerular filtration rate, a 3.1-fold decrease in the urine protein level, and a 2.5-fold decrease in protein excretion. The peptide *AEDG* reduced absolute sodium reabsorption by 1.3 times and increased distal sodium transport by 1.4 times. The realization of glomerular–tubular and tubular–tubular balances is verified by the correlation between the glomerular filtration rate (GFR) and absolute, proximal, and distal sodium reabsorption. In kidney tissue, stimulation of the antioxidant enzyme activity on the background of inhibition of the intensity peroxidation processes was observed, which, together with morphological data, reflects the absence of nephrotoxic effects. PKC and peptides *AED*, *EDL*, and *AEDG* may be considered nephroprotective agents in kidney aging.

Keywords: peptides, renal effects, nephroprotection, aging

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INTRODUCTION

According to statistical data, 10–16% of the adult population is diagnosed with kidney pathology. Every fourth man and every fifth woman over 65 years of age suffer from chronic kidney disease (CKD), the development of which is associated with an age-related reduction of kidney function and the presence of accompanying pathologies, such as hypertension, diabetes mellitus, and primary renal diseases [13, 14]. As a result, the rate of CKD development in elderly and senile-age people increases [13]. The development of CKD results in a reduced quality of life, an increased hospitalization rate, and a greater risk of cardiovascular diseases and early death [12, 19].

The timely use of preventive nephroprotective drugs, early diagnosis, and therapy for the early stages of impaired renal function development may prevent progression of the pathology and significantly decrease the complication risk [9, 10]. Study of the

changes in morphofunctional state of kidney allows the identification of potential nephroprotectors and creates conditions for their use in order to achieve the prophylactic and therapeutic effects on renal function and the water–salt metabolism.

The following peptides developed at the St. Petersburg Institute of Bioregulation and Gerontology were used in the present study: the polypeptide kidney complex (PKC) [18] and short synthetic peptides alanyl-glutamyl-aspartic acid (*AED*) [17], glutamyl-aspartyl-leucin (*EDL*) [16], and alanyl-glutamyl-aspartyl-glycine (*AEDG*) [15]. These peptides stimulate the growth of organotypic kidney tissue cultures in young and old animals and regulate the expression of signaling molecules in the primary dissociated kidney cell cultures during their aging [6–8]. The nephroprotective action of PKC and peptides *AED*, *EDL*, and *AEDG* was previously demonstrated on experimental models of toxic and ischemia/reperfusion acute kidney injury [2–4].

The goal of the present study was to study the renal effects of PKC and peptides *AED*, *EDL*, and *AEDG* in the course of their protracted administration to old rats.

MATERIALS AND METHODS

Thirty-five old (20–24 months) male and female nonlinear white rats weighing 300–350 g were used in the study. The animals were housed in a vivarium at a controlled temperature (18–22°C) and a relative humidity of 50–55%. The rats were fed a standard balanced diet (complete feed) and had ad libitum access to water. The studies were performed in accordance with the Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes [11].

The animals were randomly divided into five equal groups. The first was the control group. Groups 2–5 were inoculated intraperitoneally with peptides dissolved in water every day of the 10-day period: the PKC was injected at a dose of 300 µg/kg, and peptides *EDL* and *AED* were injected at a dose of 3 µg/kg, and peptide *AEDG* was injected at a dose of 7 µg/kg. The use of these peptide concentrations was determined in previous experiments [2–4].

The functional state of rat kidneys was examined under water loading conditions (intra-gastric administration of water in a volume of 5% of body weight followed by urine collection for 2 h). After the experiment, the animals were anesthetized with thiopental (80 mg/kg) and decapitated. To evaluate the functional state of kidneys by the photocolometry technique, the creatinine level in the blood plasma and urine was determined by Jaffe's reaction, the protein concentration in urine was determined by the reaction with sulfosalicylic acid, the sodium and potassium ion content in the blood plasma and urine was determined by flame photometry, and the contents of titratable acids and ammonium ions in the urine were determined via titration with the sodium bicarbonate solution [5]. The standardization of the indices was performed via recalculation per unit mass or per 100 µL glomerular filtrate. Based on the obtained data, the indices of kidney function (glomerular filtration rate (GFR), protein excretion, titratable acids, ammonium ions, hydrogen ions, and sodium and potassium ions, reabsorption of water and sodium ions, and proximal and distal sodium ion transport) were calculated. The levels of MDA, oxidatively modified proteins, and the activities of catalase and glutathione peroxidase in kidney homogenate were determined to assess the prooxidant-antioxidant balance [1]. Histological sections of kidneys stained with hematoxylin-eosin were studied with light microscopy (LUMAM-P8 microscope, Olympus C740UZ digital camera) and analyzed in the VideoTest–Size 5.0 software (Videotest, Russia).

Statistical data processing was performed with the SPSS Statistics 17.0 software. The data distribution in the group was determined with the Kolmogorov–Smirnov test. The significance of the differences between groups was evaluated with the Student's *t*-test (for a normal data distribution) and nonparametric Mann–Whitney *U* test (for an abnormal distribution).

The correlations between the indices were determined by the Spearman's rank correlation coefficient. The significance of the differences was assessed at $p < 0.01$ and $p < 0.05$.

RESULTS AND DISCUSSION

Effect of Polypeptide Kidney Complex on the Morphofunctional State of Kidney Tissue in Old Rats

A significant 1.2-fold increase in diuresis in animals of the experimental group as compared to the control group, which was accompanied by a trend toward increasing GFR and a decrease in water reabsorption, indicate that the PKC exhibits a diuretic action. The PKC was observed to have a hyponitrogenic action as indicated by a 1.3-fold reduction of the plasma creatinine level as compared to the control group. The PKC significantly led to a 1.8-decrease in the urine protein content and a 1.5-fold decrease in protein excretion as compared to the control group. The effect of the PKC on acid-base regulation in kidneys was expressed as a 5% increase in the urine pH, together with a significant 1.3-fold increase in the ammonium ion excretion and a lack of changes in the titratable acid excretion index. In the study of the effect of the PKC on the ion regulatory function of kidneys, a trend toward increasing urinary sodium excretion associated with increased diuresis was observed. Although no significant changes in the absolute, relative, and proximal sodium reabsorption were observed, the PKC caused a 1.2-fold increase in distal sodium transport; however the standardized index remained at the control level. No changes in the potassium excretion and the plasma potassium concentration were found in the group of animals injected with the PKC (Table 1). The state of the intrarenal autoregulation mechanisms is an important criterion for the substantive evaluation of renal function under the physiological conditions, during the development of pathology, and in the study of the renal effects of biologically active substances. In the control group, the functioning of the glomerular–tubular balance was associated with a positive correlation between GFR and absolute sodium reabsorption ($r = 0.863$), proximal sodium transport ($r = 0.893$), and distal sodium transport ($r = 0.932$). The tubular–tubular link was associated with a negative correlation between the indices of proximal and distal sodium transport ($r = -0.863$). The functioning of the glomerular–tubular balance after PKC administration was verified by the correlation between the GFR and absolute

Table 1. Index distribution in the experimental groups of rats, $M \pm SD$

Index	Control group	PKC, 300 $\mu\text{g}/\text{kg}$	EDL, 3 $\mu\text{g}/\text{kg}$	AED, 3 $\mu\text{g}/\text{kg}$	AEDG, 7 $\mu\text{g}/\text{kg}$
Diuresis, mL/2 h	3.51 \pm 0.14	4.25 \pm 0.21*	4.40 \pm 0.24*	4.78 \pm 0.29*	3.94 \pm 0.17
PCr, $\mu\text{mol}/\text{L}$	57.65 \pm 1.83	42.14 \pm 3.27*	61.36 \pm 3.70	63.38 \pm 3.66	61.70 \pm 2.88
GFR, $\mu\text{L}/\text{min}$	516.32 \pm 39.12	605.63 \pm 49.87	507.84 \pm 53.45	566.07 \pm 58.34	406.22 \pm 33.44*
RH ₂ O, %	98.19 \pm 0.40	96.94 \pm 0.49	95.32 \pm 0.79*	95.59 \pm 0.69*	94.69 \pm 0.55*
Upr, g/L	0.031 \pm 0.006	0.017 \pm 0.005*	0.019 \pm 0.005	0.011 \pm 0.003**	0.01 \pm 0.001**
Epr, mg/2 h	0.108 \pm 0.017	0.069 \pm 0.002*	0.084 \pm 0.004	0.048 \pm 0.01**	0.042 \pm 0.006*
Urine pH	7.16 \pm 0.08	7.55 \pm 0.09**	7.49 \pm 0.17*	7.13 \pm 0.14	7.40 \pm 0.12
ETK, $\mu\text{mol}/2\text{ h}$	5.87 \pm 0.47	5.95 \pm 1.02	5.79 \pm 0.59	4.47 \pm 0.58	4.93 \pm 0.59
ENH ₄ ⁺ , $\mu\text{mol}/2\text{ h}$	18.32 \pm 0.95	24.08 \pm 1.01*	16.67 \pm 1.31	16.29 \pm 0.95	21.10 \pm 2.13
ENa ⁺ , $\mu\text{mol}/2\text{ h}$	1.36 \pm 0.14	2.11 \pm 0.52	4.38 \pm 0.16**	2.20 \pm 0.42**	1.66 \pm 0.14
RNa ⁺ , $\mu\text{mol}/\text{min}$	85.61 \pm 7.50	99.58 \pm 7.76	86.01 \pm 8.55	87.56 \pm 7.30	66.42 \pm 5.72*
RfNa ⁺ , %	98.87 \pm 0.12	98.56 \pm 0.18	98.49 \pm 0.21	98.17 \pm 0.35**	98.62 \pm 0.11
TpNa ⁺ , mmol/min	67.33 \pm 6.15	78.12 \pm 6.39	66.50 \pm 7.15	67.80 \pm 6.11	50.89 \pm 4.64*
TdNa ⁺ , $\mu\text{mol}/\text{min}$	3.99 \pm 0.19	4.87 \pm 0.29*	5.18 \pm 0.20**	5.16 \pm 0.27**	4.46 \pm 0.23
TdNa ⁺ , $\mu\text{mol}/100\ \mu\text{L}$	0.79 \pm 0.01	0.83 \pm 0.08	1.09 \pm 0.12*	0.97 \pm 0.10*	1.13 \pm 0.08**
PK ⁺ , mmol/L	5.96 \pm 0.36	5.93 \pm 0.38	5.18 \pm 0.25	5.54 \pm 0.36	5.89 \pm 0.18
EK ⁺ , $\mu\text{mol}/2\text{ h}$	22.61 \pm 2.85	21.66 \pm 2.20	24.31 \pm 2.83	19.90 \pm 2.40	21.79 \pm 2.28

PCr—creatinine concentration in the blood plasma; RH₂O—water reabsorption; Upr—protein concentration in urine; Epr—protein excretion; ETK—excretion of titratable acids; ENH₄⁺—excretion of ammonium ions; ENa⁺—excretion of sodium ions; RNa⁺—absolute sodium reabsorption; RfNa⁺—relative sodium reabsorption; TpNa⁺—proximal sodium transport; TdNa⁺—distal sodium transport; PK⁺—potassium concentration in the blood plasma; EK⁺—potassium excretion; here and in Table 2: * $p < 0.05$, ** $p < 0.01$ —statistically significant differences from the control group.

sodium reabsorption ($r = 0.893$) and proximal sodium transport ($r = 0.821$); the tubular–tubular link remained negative ($r = -0.739$), as in the control group.

On the histological kidney sections, no pathological changes in the morphological organization of tissue were observed: the glomerular architecture was normal, and the epithelium of the proximal and distal tubules was without pathological alterations. Signs of reverse hydropic degeneration were observed in 3% of epithelial cells, the tubular space was free, and the interstitium was normal (Fig. 1). Reverse hydropic swelling was observed in 9% of the epithelial cells of proximal kidney tubules, primarily in the juxtamedullary zone, in the kidneys of rats administered with the PKC. This is within the physiological age norm (Fig. 2a). These changes may be associated with the influence of the PKC on the transport of water, protein, and ions in the proximal kidney tubules.

Effect of the Peptide EDL on the Morphofunctional State of Kidney Tissue of Old Rats

The peptide EDL exhibited a diuretic effect verified by a 1.3-fold increase in diuresis as compared to the

control group (Table 1). The peptide did not affect the creatinine concentration in the blood plasma and GFR but led to a 2.9% reduction of water reabsorption, which reflects the tubular mechanism of diuresis

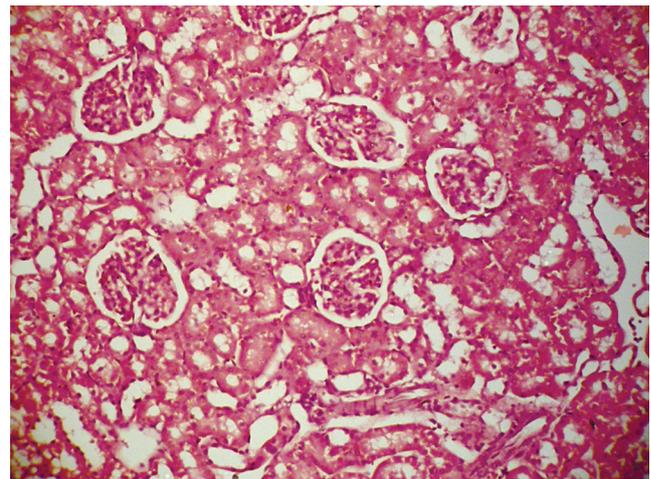


Fig. 1. Preparation of the kidney cortex of the rat in the control group. Hematoxylin and eosin stain; magnification, 100 \times .

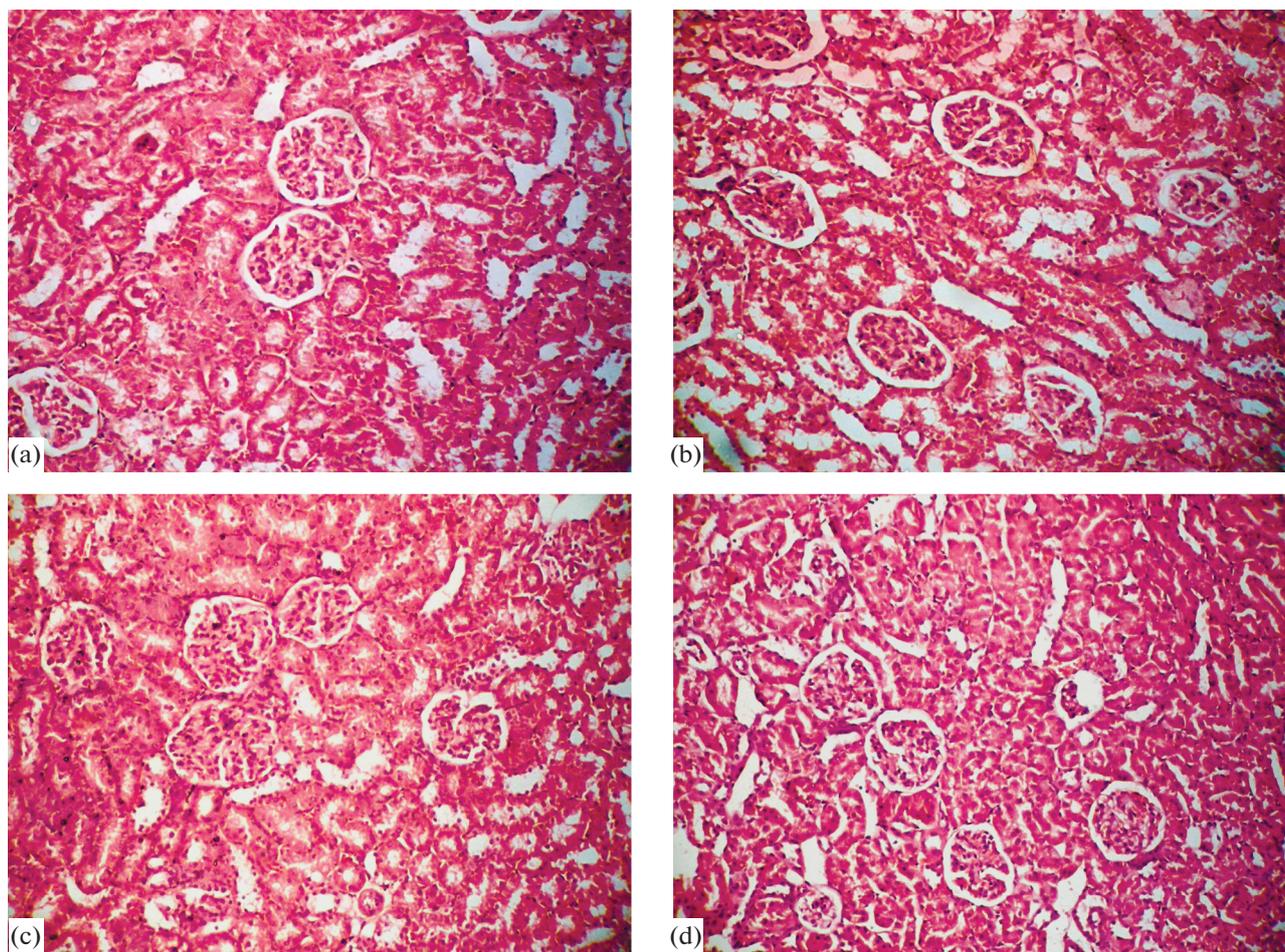


Fig. 2. Preparation of the kidney cortex of the rat after administration of peptides: (a) polypeptide kidney complex; (b) *EDL*; (c) *AED*; (d) *AEDG*. Hematoxylin and eosin stain; magnification, 100 \times .

increase ($r = -0.571$). In the group of animals injected with *EDL*, a downward trend in the urine protein concentration and protein excretion was observed, although the difference from the control group was not statistically significant. The effect of *EDL* on the ion-regulating kidney function was expressed by a 1.3-fold increase in the sodium excretion. At the same time, the indices of absolute and relative reabsorption and proximal sodium transport remained unchanged; the significant correlation with the GFR ($r = 0.964$) reflects normal functioning of the glomerular–tubular balance after peptide administration. Although there was no effect of the peptide *EDL* on the proximal sodium transport, the distal transport increased by 1.3 times, which is confirmed by the increase in the index standardized against GFR and the tubular–tubular balance associated with the negative correlation ($r = -0.893$) between the transport indices in different nephron regions. Histological analysis found signs of hydropic swelling of the individual epithelial cells in proximal kidney tubules (Fig. 2b), which corresponds to physiological age-related changes.

Effect of Peptide AED on the Morphofunctional State of Kidney Tissue of Old Rats

The peptide *AED* caused a 1.4-fold increase in diuresis via a 2.6% reduction of tubular water reabsorption ($r = -0.758$) accompanied by a lack of changes in the GFR and the blood creatinine level (Table 1). A significant effect on protein transport was observed: in the group of animals receiving peptide *AED*, the protein concentration in urine decreased significantly by 2.8 times and the protein excretion decreased by 2.3 times. A decreasing tendency was observed in the excretion of the titratable acids and ammonium ions after the administration of the peptide *AED*, whereas no effect on the urine pH was demonstrated. The treatment with the *AED* led to a 1.6-fold increase in the urinary sodium excretion, which was associated with increased diuresis. The absolute sodium reabsorption did not change, although the relative reabsorption index decreased, which led to a 1.3-fold increase in distal sodium transport via tubular–tubular balance. The *AED* did not affect the potassium transport and plasma potassium

Table 2. Index distribution in the experimental groups of rats, $M \pm SD$

Group	Glutathione peroxidase activity, nmol/(min mg)	Catalase activity, $\mu\text{mol}/(\text{min mg})$	MDA, $\mu\text{mol/g}$	Oxidative modification of proteins, U/g
Control	109.33 \pm 6.33	7.74 \pm 0.30	29.72 \pm 0.57	11.43 \pm 0.32
PKC	135.75 \pm 6.79*	8.79 \pm 0.26*	29.23 \pm 0.87	11.04 \pm 0.50
EDL	169.91 \pm 14.38**	8.76 \pm 0.19*	25.49 \pm 0.60**	10.16 \pm 0.39*
AED	145.14 \pm 14.2*	8.04 \pm 0.38	17.47 \pm 2.49**	11.47 \pm 0.50
AEDG	142.35 \pm 9.31*	8.69 \pm 0.46*	27.03 \pm 0.32**	12.23 \pm 0.42

concentration. The maintenance of the glomerular–tubular balance after *AED* injection is characterized by the correlation between the GFR and absolute sodium reabsorption and proximal sodium transport ($r = 0.969$). The tubular–tubular balance functioning is verified by the negative correlation between the proximal and distal sodium transport ($r = -0.893$). The study of the histological preparation of the kidney tissue demonstrated the correspondence of the morphological pattern to the age norm, which is accompanied by the absence of pathological changes in the structures of glomeruli, tubular epithelium, and interstitium (Fig. 2c).

Effect of the Peptide AEDG on the Morphofunctional State of Kidney Tissue of Old Rats

The peptide *AEDG* decreased the GFR by 21.3%, while the blood creatinine concentration remained unchanged (Table 1). After *AEDG* administration, diuresis remained at the control level and the water reabsorption decreased by 1.5% as compared to the control group. The peptide exhibited the most pronounced effect on tubular protein transport: the protein concentration in urine decreased by 3.1 times and protein excretion decreased by 2.5 times as compared to intact animals. The peptide did not affect acid-base regulation and potassium transport. A decrease in the GFR in response to *AEDG* injection led to a 1.3-fold reduction in the absolute sodium reabsorption due to the corresponding decrease in the transport activity in the proximal nephron. The activation of the tubular–tubular balance contributed to the increase in the distal sodium transport as indicated by the 1.4-fold increase in the standardized index. The correlation between the GFR and absolute sodium reabsorption ($r = 0.964$), proximal sodium transport ($r = 0.929$), and the proximal and distal sodium reabsorption ($r = -0.75$) reflects the realization of glomerular–tubular and tubular–tubular balance. The histological study found dystrophic changes, such as hydropic swelling of the 5% of the epithelial cells in proximal tubules, which is within the age norm (Fig. 2d).

Effect of Peptides on the Prooxidant–Antioxidant Balance in the Kidney Tissue of Old Rats

An important component of homeostasis maintenance is the constancy of the prooxidant–antioxidant balance in the body. The peroxidation processes are necessary for normal functioning of the biosystems and the regulation of the physicochemical properties of cell membranes. Reactive oxygen species play the role of secondary messengers in the cellular metabolism in the process of intercellular and intracellular regulatory signal transduction, whereas peroxidation intensification leads to the development of oxidative stress involved in the pathogenesis of various diseases. The study of the influence of the protracted administration of peptides on the indices of prooxidant–antioxidant balance in rat kidneys made it possible to reveal their tissue-specific effects (Table 2).

The oligopeptides *EDL*, *AED*, and *AEDG* caused a decrease in lipid peroxidation, which was accompanied by a decrease in the content of oxidatively modified proteins in response to *EDS* administration. Among the effects on the antioxidant system, an increase in the catalase activity was observed in the group of animals treated with the PKC and the peptides *EDL* and *AEDG*, whereas an increase in the glutathione peroxidase activity was demonstrated in all groups as compared to the control, with the most pronounced effect observed after *EDS* injection.

CONCLUSIONS

Based on the experimental data, it may be concluded that, in a 10-day course of protracted treatment of old animals, all of the examined peptides (PKC, *EDL*, *AED*, and *AEDG*) affect the processes of the tubular transport of water, protein, and ions in the nephrons, whereas the intrarenal autoregulation mechanisms remain intact. In addition, the polypeptide kidney complex and peptides *AED* and *EDL* exhibit a mild diuretic effect. The lack of significant shifts in the prooxidant–antioxidant homeostasis and pathological alterations in the histological structure of kidneys indicate that these peptides do not exhibit nephrotoxic properties. The results of the study demonstrate the reliability and promise of the use of

the PKC and short peptides *EDL*, *AED*, and *AEDG* for the prophylaxis of kidney pathology in aging.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflict of interest.

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The studies were performed in accordance with the Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes

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