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Original medicinal plant collection reduces nephrotoxicity of gentamicin in rats

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Abstract
The nephroprotective properties of the infusion (1:10) of the original medicinal plant collection containing Polygoni avicularis herba, Fragariae herba, Juglandis folium, Uvae ursi folia, Urticae folia, Elymi repensis rhizomata et radices (25,0 for each) and Helichrysi arenarii flores (20,0), has been estimated on the model of gentamicin-induced renal injury in rats. It has been found out that the infusion possesses hypoazotemic, antiproteinuric activity under the water-loading test. The effects of the infusion at a dose of 5 ml/kg and 10 ml/kg were comparable with that of the comparison drug "Chophytol" at a dose of 100 mg/kg.

Keywords: medicinal plant collection, infusion, kidney, gentamicin, nephroprotective agents, rats

1. Introduction
The antibiotics of aminoglycoside group take the leading place among the drugs that cause acute tubular necrosis. It is known that nephrotoxicity depends on the amount of amino groups in the antibiotic molecule and increases with increasing their quantity. Neomycin, gentamicin, kanamycin and tobramycin are the most toxic among them [6, 11]. The frequency of nephrotoxic reactions on the base of usage aminoglycosides can reach 10-20%. Despite the fact that the commonly induced aminoglycoside kidney damage is reversible, it prolongs hospitalization and increases the cost of patients' treatment. Herbal medicines containing substances with antioxidant activity can be successfully used for the prophylaxis or treatment of gentamicin-induced nephropathy [10, 12]. Prevention of aminoglycosides' nephrotoxicity can be oriented to avoid damaging the membranes of lysosomes and mitochondria, reducing the generation of free radicals. Extracts of medicinal plants that have antioxidant activity can improve the functional condition of the kidneys, decreasing the nephrotoxic effect of aminoglycosides [10, 12]. This study is a fragment of the research of the original medicinal plants' collection, containing Polygoni avicularis herba, Fragariae herba, Juglandis folia, Uvae ursi folia, Urticae folia, Elymi repensis rhizomata et radices (25,0 for each) and Helichrysi arenarii flores (20,0), for which a diuretic, antioxidant and nephroprotective activity had been found out under the conditions of gentamicin-induced acute renal failure [4, 8].
The aim of the work is to determine the effectiveness of the plant collection of original composition under the conditions of gentamicin-induced nephropathy.

2. Materials and Methods
The experiments were conducted on sexually mature female rats (160-280 g) according to bioethics requirements. During the experiment animals were kept in standard conditions of the vivarium with free access to water and food. Gentamicin sulfate solution («Halychpharm», Ukraine) at a dose of 80 mg/kg was administered intramuscularly once a day during a week to the rats of the model pathology and experimental groups [5]. The rats of the intact control group received an equivalent amount of 0.9% sodium chloride solution in **a**n analogous regime. During the course of the gentamicin introduction, The animals of the studied groups received infusion (1:10) of the original plant collection (dosage - 5 ml / kg or 10 ml / kg) intragastrically and the drug of comparison "Chophytol" (Laboratories Rosa-Phytopharma, France, tablets) - a herbal medicine with already experimentally confirmed nephroprotective activity [2] at a dose of 100 mg / kg. "Chophytol" dose is calculated taking into account the coefficients of species sensitivity and corresponds to a daily therapeutic dose for humans. The division of the animals into groups is given below:

1. Intact control (n = 7);
2. Model pathology (n = 7);
3. Gentamicin + plant collection infusion, 5 ml / kg (n = 6);
4. Gentamicin + plant collection infusion, 10 ml / kg (n = 6);
5. Gentamicin + "Chophytol", 100 mg / kg (n = 6).

Water diuresis, induced by intragastric administration of water of room temperature in the volume of 3% of body weight, was caused on the seventh day of drugs' administration in the rats, previously adapted to the experimental conditions. The urine was collected during 2 hours. After that, the animals were decapitated under thiopental-sodium anesthesia, blood was collected and received serum. Kidneys and liver were extracted and were determined their weight coefficients. The creatinine content was measured by the Jaffe reaction, urea - with diacetylmonooxime, sodium and potassium by the method of flame photometry in urine and blood serum samples; protein content by reaction with sulfosalicylic acid in urine. The excretion of these compounds was determined. In addition, the percentage of water loading removal, glomerular filtration rate (GFR), filtration charge of sodium, reabsorption of sodium and water, urea clearance, sodium / potassium coefficient of urine and blood serum were calculated.
The statistical significance of intergroup differences was estimated by the U Mann-Whitney criterion, the relationship between individual indices within the group - by means of correlation analysis (Spirman correlation coefficient).

3. Results and Discussion
Nephrotoxicity of gentamicin was verified by increasing the concentration of creatinine and urea in blood serum (45.7% and 49.3% respectively), increased proteinuria, changes in the partial renal function (Table 1, 2). There was a significant ($p<0.05$) reduction of tubules water reabsorption compared with intact control group under conditions of water diuresis. Despite the fact that the GFR decreased a little, tubular transport disturbances were manifested in the tendency to diuresis increasing. Dissociation between the reducing of tubular water reabsorption and the lack of changes in sodium reabsorption may indicate a violation of the antidiuretic hormone dependent water transport, indicating the damage of distal nephron and, in particular, collecting tubules. The concentration of protein in the urine increased two times more ($p<0.005$) and its excretion - 2.4 times ($p<0.05$), indicating damage of the tubular epithelium, hence it is one of the important factors of kidney disease progression.
The water reabsorption increased to the physiological norm under the influence of the plant collection infusion. Also, the infusion, eliminating the imbalance between this process and sodium reabsorption, inherent in animals of the model pathology group, dose-dependent reduced proteinuria, not inferior the effectiveness of the comparison drug. The studied infusion at a dose of 10 ml / kg countered the increasing of retention azotemia: the concentration of creatinine and urea in serum did not significantly differ from that in the intact control group. The infusion at a dose of 5 ml / kg and "Chophytol" also effectively lowered hypercreatininemia, but less affected the serum urea level. The negative correlation between the GFR and serum creatinine level indicates about the involvement of renal mechanisms to overcome hyperazotemia on the basis of usage the infusion (10 ml / kg) and "Chophytol". The Spirman correlation coefficient is respectively -0.89 ($p<0.05$) and -0.78 ($p<0.05$) versus -0.79 ($p<0.05$) in intact animals. This ratio was equal to -0.14 ($p<0.05$) and -0.23 ($p<0.05$) for the model pathology and plant collection infusion (5 ml / kg) groups, which indicates a disturbance of the renal mechanisms of azotemia control.
Urea excretion was tended to be decreased in comparison with the intact animals and urea clearance was significantly lowered ($p<0.05$) on the basis of treatment with study infusion. This may indicate about the involvement of nonrenal mechanisms for hyperazotemia overcoming.
Hypokalemia is a characteristic manifestation of gentamicin-induced nephropathy both in the experiment and in the clinic [9]. As can be seen from Table 3, the serum potassium concentration was 4.19 ± 0.28 mmol / l in rats of the model-pathology group versus 5.30 ± 0.38 mmol / L for intact animals ($p<0.05$). The plant collection infusion normalized the concentration of blood potassium (in a dose of 5 ml / kg it was 5.08 ± 0.46 mmol / l, in a dose of 10 ml / kg - 5.14 ± 0.78 mmol / l), the effect of "Chophytol" on hypokaliemia was less significant (4.78 ± 0.60 mmol / l). Water-electrolyte disturbances such as hypovolemia, potassium and magnesium deficiency may complicate the course of gentamicin nephropathy and lead to the progression of kidney's functional disorders caused by aminoglycosides [6]. It has been shown that potassium administration causes a protective effect under the condition of gentamicin nephropathy, which is associated with stimulation of Na+, K+ -ATPase activity in the renal cortex and with the decreasing accumulation of gentamicin in the kidneys [7]. Probably, the plant collection infusion posseses similar properties, which may be due to the sufficient content of potassium and magnesium in medicinal plants that are part of it [3]. The level of blood serum sodium was comparable in all groups of animals, sodium / potassium coefficient was without significant changes. Intergroup differences of sodium and potassium renal excretion were not reliable. The excretion of these electrolytes was significantly higher only in rats receiving the infusion in a dose 10 ml / kg than the same index on the basis of "Chophytol" administration. The low natriuresis in comparison with kaliuresis (Table 3), that is evidenced via the ratio of sodium and potassium concentrations in urine and can be explained by the peculiarities of the rats' diet, particularly feeding the animals with wheat grain containing large quantities of potassium. The latter stimulates the secretion of aldosterone, which acts on sodium retention. Sodium filtration charge and its reabsorption were not significantly different in all studied groups. Changes of kidney and liver weight coefficients were also absent. Thus, the infusion of medicinal plant collection possesses a nephroprotective effect under conditions of gentamicin-induced nephropathy. Diuretic and hypoazotemic properties of herbal medicine may be involved in its implementation.

Table 1: Infuence of plant collection infusion and "Chophytol" on excretory renal function in ratswith gentamicin nephropathy under the conditions of water diuresis (M±m)

The conditions of the experiment, study drugs	Intact control (n=7)	Model pathology (gentamicin) (n=7)	Gentamicin + plant collection infusion, 5 ml / kg (n=6)	Gentamicin + plant collection infusion, 10 ml / kg (n=6)	Gentamicin + "Chophytol", 100 mg / kg (n=6)
Diuresis, ml/100 g for 2 hours	2,43±0,23	2,78±0,28	1,90±0,31$^{\#}$	2,60±0,10	2,19±0,19
Water loading excretion,%	81,0±7,7	92,6±9,2	63,4±10,2$^{\#}$	86,8±3,4	74,5±5,9
GFR, ml/min on 100 g	0,072±0,007	0,064±0,010	0,054±0,008	0,070±0,007	0,071±0,011
Na^+ filtration charge, mM/min on 100 g	11,1±1,1	10,1±1,7	8,3±1,1	10,9±1,1	11,2±1,8
Na^+ reabsobtion,%	99,97±0,01	99,98±0,01	99,97±0,01	99,98±0,004	99,99±0,003
Water reabsorbtion,%	97,5±0,3	96,7±0,4*	97,4±0,4	97,3±0,3	97,7±0,2
Concentration of protein in urine, g/l	0,035±0,002	0,071±0,009***	0,060±0,008***	0,051±0,004	0,053±0,006
Protein excretion, mg/100 g for 2 hours	0,084±0,006	0,198±0,036*	0,111±0,026	0,134±0,014	0,118±0,008
Na^+ excretion, mM/100 g for 2 hours	5,12±2,72	2,30±0,91	3,38±1,51	3,83±0,57$^{\wedge}$	1,84±0,53
K^+ exretion, mM/100 g for 2 hours	33,4±6,2	38,3±7,3	25,5±7,4	38,0±2,5$^{\wedge}$	24,4±4,0
Coefficient of sodium / potassium of the urine	0,13±0,06	0,06±0,02	0,11±0,03	0,10±0,02	0,07±0,01
Creatinine excretion, mM/100 g for 2 hours	4,79±0,39	6,18±1,04	4,62±0,90	5,79±0,47	4,75±0,44
Urea excretion, m/100 g for 2 hours	167,8±12,3	172,9±23,3	120,7±17,2	147,0±11,5	141,6±17,3
Urea clearance, ml/min on 100 g	0,42±0,05	0,30±0,04	0,24±0,06*	0,28±0,02*	0,26±0,07*

Note. Significant differences: with intact control group – * (p<0,05), *** (p<0,005); model pathology group – $^{\#}$ (p<0,05); gentamicin + "Chophytol" group – $^{\wedge}$ (p<0,05); n – number of animals in the group.

Table 2: Infuence of plant collection infusion and "Chophytol" on the concentration of blood serum creatinine and urea in rats with gentamicin nephropathy (M±m)

The conditions of the experiment, study drugs	Blood serum concentration	
	Creatinine, mmmol/l	Urea, mmol/l
Intact control (n=7)	47,1±1,1	3,43±0,28
Model pathology (gentamicin) (n=7)	68,6±4,1***	5,12±0,58*
Gentamicin + plant collection infusion, 5 ml / kg (n=6)	53,4±6,6	4,56±0,54*
Gentamicin + plant collection infusion, 10 ml / kg (n=6)	61,1±9,7	4,40±0,32
Gentamicin + "Chophytol", 100 mg / kg (n=6)	53,8±4,9	5,20±0,75*

Note. Significant differences: with intact control group – * (p<0,05), *** (p<0,005); n – number of animals in the group.

Table 3: Infuence of plant collection infusion and "Chophytol" on blood serum sodium and potassium levels with gentamicin nephropathy (M±m)

The conditions of the experiment, study drugs	Blood serum concentration, mmmol/l		Coefficient of blood serum sodium / potassium
	sodium	potassium	
Intact control (n=7)	156,2±4,0	5,30±0,38	30,7±2,8
Model pathology (gentamicin) (n=7)	157,5±3,9	4,19±0,28*	38,7±2,8
Gentamicin + plant collection infusion, 5 ml / kg (n=6)	154,5±3,2	5,08±0,46	31,7±3,0
Gentamicin + plant collection infusion, 10 ml / kg (n=6)	156,4±2,2	5,14±0,78	33,5±5,2
Gentamicin + "Chophytol", 100 mg / kg (n=6)	158,0±1,3	4,78±0,60	36,1±4,8

Note. Significant differences: with intact control group – * (p<0, 05), n – number of animals in the group.

4. Conclusions

1. Infusion of the original plant collection, containing Polygoni avicularis herba, Fragariae herba, Juglandis folium, Uvae ursi folia, Urticae folia, Elymi repensis rhizomata et radices (25,0 for each) and Helichrysi arenarii flores (20,0), at doses of 5 and 10 ml / kg during seven day couse administration causes dose-dependent nephroprotective effect on the model of gentamicin-induced nephropathy in rats.
2. The infusion of the investigated plant collection eliminates the reduction of water tubular reabsorption, decreases the severity of proteinuria, azotemia, prevents the development of hypokalemia, not inferior to the effectiveness the drug of comparison "Chophytol" at a dose of 100 mg / kg.

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