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FUNCTIONAL KIDNEY STATE OF MATURE RATS WITH RAPID ACETYLATION TYPE UNDER CONDITIONS OF SUBACUTE CADMIUM-NITRATE INTOXICATION AND PREVENTIVE INTRODUCTION OF *ECHINACEA PURPUREA*

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Abstract

The objective of the study was to investigate the changes of the kidney functions in mature rats with rapid acetylation type, under conditions of subacute cadmium-nitrate intoxication and preventive introduction of *Echinacea purpurea*.

Methods. Male rats, 6 months of age, with rapid acetylation type, were divided into 3 groups: I – control; II – rats with of subacute cadmium-nitrate intoxication model; III – rats receiving intragastric alcohol tincture of fresh rhizomes with roots of *Echinacea purpurea* (LC „State Municipal Enterprise «Pharmaceutical Plant Vishpha»", Ukraine; 0,25 mk/rg).

Results. Under the effect of preventive introduction of *Echinacea purpurea* in rats with rapid acetylation type and cadmium-nitrate lesion of the kidneys, diuresis and glomerular filtration rate became normal; distal transport of sodium ions increased; hyponatraemia and decreased natriuresis remained without changes; concentrations of creatinine in the blood plasma, potassium ions in the blood plasma and urine,

Résumé

L'état de la fonction des reins des rats sexuellement matures de type rapide d'acétylation dans les conditions d'intoxication sous-aiguë de cadmium-nitrate et d'introduction préventive d'E*chinacea purpurea*

Le but de l'étude. Étudier les changements dans la fonction rénale des rats sexuellement matures des acétylateurs rapides avec l'intoxication aiguë de cadmium-nitrate en conditions de l'introduction préventive d'*Echinacea purpurea*.

Méthodes. Des rats mâles de 6 mois avec un type d'acétylation rapide étaient divisés en 3 groupes: I – de contrôle; II – des rats avec le modèle d'intoxication aiguë de cadmium-nitrate; III – des rats auxquels une heure et demie avant la modélisation était introduite, par la voie intragastrique (i/g), la teinture alcoolique de rhizomes frais avec des racines d'*Echinacea purpurea* (Ltd «DKP» usine pharmaceutique Vishpha «, Ukraine; 0,25 ml/kg).

Résultats. Sous l'influence de l'administration prophylactique d'Echinacea purpurea chez des rats

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kaliuresis decreased. Similar to the group of rats without *Echinacea purpurea,* proximal reabsorption of sodium ions, which is standardized by glomerular filtration, decreased.

Conclusion. Under conditions of preventive introduction of *Echinacea purpurea,* volume regulating kidney function is maintained in mature rats with rapid acetylation type and subacute cadmium-nitrate intoxication, but it does not prevent the development of toxic nephropathy, with prevailing lesion of the nephron proximal portion, disorders of ion regulating kidney function and water-electrolytic balance.

Keywords: *Echinacea purpurea*, mature rats, rapid acetylation type, cadmium-nitrate intoxication, kidney functions.

Abbreviations: SCNI – subacute cadmium-nitrate intoxication, GFR – glomerular filtration rate.

présentant une acétylation rapide avec des lésions rénales dues au nitrate de cadmium, la diurèse et la vitesse de filtration glomérulaire sont revenues à la normale; le transport distal des ions de sodium augmente; l'hyponatrémie reste inchangée, l'uréase de sodium est abaissée; les concentrations de créatinine dans le plasma sanguin, des ions de potassium dans le plasma et l'urine, de l'uréase du potassium diminuent. Comme dans le groupe des rats sans *Echinacea purpurea*, il y a une diminution de la réabsorption proximale des ions de sodium normalisée pour la filtration glomérulaire.

Conclusions. En les conditions de l'introduction préventive, l'*Echinacea purpurea* soutient la fonction volumétrique des reins des rats sexuellement matures des acétylateurs rapides avec l'intoxication aiguë de cadmium-nitrate, cependant, elle n'empêche pas le développement d'une néphropathie toxique avec des dommages prédominants au néphron proximal, la violation de la fonction de régulation ionique des reins et de l'équilibre eau-électrolyte.

Mots-clés: *Echinacea purpurea*, des rats sexuellement matures, un type d'acétylation rapide, intoxication de cadmium-nitrate, la fonction des reins.

Introduction

Contemporary medicine is in search of directions for effective body protection against a combined effect of environmental xenobiotics, which is a threat for a living organism. The attention of clinicians and scientists was always drawn to phytotherapeutic remedies, since the majority of medications of plant origin has antioxidant, anti-hypoxanthine, immune modulating, anti-inflammatory, and antineoplastic action. This pharmacodynamics spectrum is specific for the remedies on the base of *Echinacea purpurea*. A considerable amount of information is indicative of a certain interest of researchers to protective properties of *Echinacea purpurea*, including those manifested in case of toxic body damage[1-4]. At the same time, reports concerning *Echinacea purpurea* present different views, the authors emphasize the dependence of the efficacy of these remedies on human age, indicate its organ toxicity, especially in case of self-treatment[5-8].

When *Echinacea purpurea* tincture is introduced as a preventive measure, reactions of the liver antioxidant system against the ground of cadmium-nitrate intoxication are known to manifest differently in rats with various acetylation type[9]. Such environmental pollutants, as cadmium chloride and sodium nitrate, are powerful nephrotoxins[10-11]. Considering the importance of the issue on kidney protection, the relations between renal effects of preventive administration of the remedies on the base of *Echinacea purpurea* and acetylation phenotype are of certain interest.

The aim of the study was to investigate the changes of the kidney functions in mature rats with rapid acetylation type, under conditions of subacute cadmium-nitrate intoxication and preventive introduction of *Echinacea purpurea*.

Materials and methods

The study was conducted on laboratory non-linear albino male rats, 6 months of age, with the body weight of 0.16-0.18 kg, kept under standard vivarium conditions on balanced feeding. Acetylation ability of rats was determined according to certain methods[12]. Rats with a quick type of acetylation were divided into 3 groups: I – control; II – rats with of subacute cadmium-nitrate intoxication (SCNI) model; III – rats receiving intragastric, alcohol tincture of fresh rhizomes with roots of *Echinacea purpurea* (LC State Municipal Enterprise „Pharmaceutical Plant Vishpha", Ukraine; 0.25 mk/rg). To simulate the model, rats were injected with cadmium chloride during 14 days intraperitoneally in the dose of 0.1 mg/kg (1/150 DL_{50}) and sodium nitrate intragastric (through the probe) in the dose of 500 mg/kg (1/15 DL_{50}). During the whole period of the experiment, the control group was injected with isotonic

solution of sodium chloride (intraperitoneally) and settled tap water (intragastric), instead of external poisonous substances. 24 hours after the last introduction, all the rats were subjected to 5% water load (tap water, intragastric) and placed into exchange cages to collect urine. Diuresis was registered every 2 hours, blood was taken in the amount of 20 mg/kg from rats narcotized by means of 1% of sodium ethaminal solution. The concentration of sodium ions in urine and blood plasma was determined by means of flame photometry method; creatinine in urine – by means of Folin's method, in the blood plasma – by means of Poper's method in Merson's modification[13]. Parameters of the kidney function, standardized by the body mass and glomerular filtrate volume, were calculated by the common formulas[14].

Table 1. Indices of functional kidney state of mature rats with rapid acetylation type, under conditions of subacute cadmium-nitrate intoxication and preventive introduction of *Echinacea purpurea* (M±m, n=7).

Indices	*Control*	*SCNI*	*SCNI* + **Echinacea purpurea**
Diuresis, mL/2 hour	3.3±0.19	3.8±0.09 p<0.05	3.7±0.15
Concentration of Na^+ in urine, mmol/L	2.30±0.114	0.74±0.154 p<0.001	0.62±0.064 p<0.001
Excretion of Na^+, micromole/2 hour	7.7±0.62	2.8±0.54 p<0.01	2.3±0.28 p<0.001
Concentration of Na^+ in blood plasma, mmol/L	136.8±1.87	127.8±0.88 p<0.01	127.1±0.85 p<0.01
Concentration of K^+ in urine, mmol/L	16.8±1.87	9.7±1.36 p<0.01	6.4±0.49 p<0.01; p_1<0.05
Excretion of K^+, micromole/2 hour	57.4±8.47	36.3±4.71 p=0.05	23.7±1.89 p<0.01; p_1<0.05
Concentration of K^+ in blood plasma, mmol/L	20.7±0.56	19.5±0.35	18.86±0.37 p<0.05
Creatinine concentration in urine, mmol/L	0.92±0.041	1.07±0.059	0.86±0.064 p_1<0.05
Creatinine concentration in blood plasma, micromole/L	69.3±0.60	69.6±0.56	66.0±1.49 p_1<0.05
Glomerular filtration rate, microliter/min	369.8±23.23	485.1±26.20 p<0.05	402.9±34.16
Creatinine excretion, micromole/2 hour	3.1±0.19	4.0±0.22 p<0.01	3.2±0.25 p_1<0.05
Relative reabsorption of H_2O, %	92.4±0.30	93.4±0.38	92.1±0.71
Filtration fraction of Na^+, micromole/min	50.4±2.68	61.9±3.36 p<0.05	51.3±4.56
Excretory fraction of Na^+, micromole/min	0.064±0.0052	0.023±0.0045 p<0.01	0.019±0.0024 p<0.001
Clearance Na^+, mL/2 hour	0.03±0.005	0.02±0.004 p<0.01	0.02±0.002 p<0.01
UNa^+/UK^+, units	0.15±0.018	0.07±0.010 p<0.01	0.10±0.013 p<0.05
Clearance of H_2O Na^+, mL/2 hour	3.3±0.18	3.8±0.09 p<0.05	3.7±0.15
Distal reabsorption of Na^+, micromole/2 hour	447.9±21.15	481.5±12.47	466.5±20.46
Proximal reabsorption of Na^+, mmol/2 hour	5.6±0.31	6.9±0.40 p<0.05	5.7±0.54
Distal reabsorption of Na^+, micromole/100 microliter GF	1.02±0.044	0.84±0.050 p<0,05	1.00±0.089
Proximal reabsorption of Na^+, mmol/100 microliter GF	12.6±0.17	11.9±0.09 p<0.01	11.7±0.13 p<0.01

Notes: SCNI – subacute cadmium-nitrate intoxication; p – reliability in comparison with the control rats; n – number of rats in every group; GF – glomerular filtration.

The findings were statistically processed by means of the program «Statgrafics», consistency of data was assessed by Student t-criterion.

Results

Under SCNI conditions in mature rats with rapid acetylation type, the functional state of the kidney changed (Table 1). The volume regulating and excretory kidney function activated, glomerular filtration rate (GFR) and proximal transport of sodium ions per 2 hours increased, in spite of decreased excretion of sodium ions with urine, the concentration of sodium ions in the blood plasma decreased considerably[15]. The analysis of the tubular processes, standardized by glomerular filtration, determined a 5.6 and 7.7 % decreased reabsorption of sodium ions in the proximal and distal nephron segments. Kaliaemia level did not change. The concentration of potassium ions in urine decreased by 42.3 %, and kaliuresis by 36.8%. The concentration ratio of sodium and potassium ions in urine was 2.1 times less than that of intact rats.

In rats with SCNI, who previously received *Echinacea purpurea* tincture, the indices of water-salt metabolism were characterized by a restored control level of diuresis and water free from sodium ions. The concentration of sodium ions in blood plasma did not change, and was 7.1% less than that in intact rats. The concentration of sodium ions in urine and natriuresis remained lower than in the control group, and differences were 73.1 and 71.2 %, respectively. Distal reabsorption and tubular transport of sodium ions calculated for 2 hours was within the normal limits. However, only the index of distal reabsorption standardized by glomerular filtration was on the level of the control group, and the level of proximal reabsorption was lower, similar to that of the group without the remedy.

Echinacea purpurea produced a certain effect on potassium balance. Kaliaemia decreased by 8.9% in comparison with the intact group. Compared with rats under SCNI, a greater tendency to decreased concentration of potassium ions in urine and kaliuresis was admitted. The above indices were 61.9 and 58.7% less than that of the control, and 34.1 and 34.8% less than that in the group without preventive introduction of the tincture.

Further assessment of the kidney functional state demonstrated that, after administration of *Echinacea purpurea,* development of cadmium-nitrate damage of the organism of rats was not associated with GFR activation. The indices of the kidney excretory function, such as concentrations of creatinine in urine, blood plasma and its excretion, achieved the control level due to decreased values to 19.7%, 5.2% and 20%, respectively. It should be noted that a decreased creatinine level in blood plasma in rats with cadmium-nitrate damage of the body is indicative of corrective opportunities of *Echinacea purpurea,* under conditions of retention azotemia.

Discussion

The results obtained indicate that preventive administration of *Echinacea purpurea* changes kidney reaction in rats with rapid acetylation type, in response to SCNI. The relative water reabsorption in rats of all the groups did not differ. Therefore, diuresis similar to the indices of excretory kidney function was restored in accordance with GFR dynamics. At the same time, decreased diuresis and excretion of water free from sodium ions could result from stimulating effect of *Echinacea purpurea* on the central nervous system and vasopressin activation. The above changes are likely to demonstrate the *Echinacea purpurea* participation in volume regulation and maintenance of extracellular fluid volume of the kidneys damaged by exotoxins.

After preventive administration of the tincture, filtration fraction of sodium ions decreased. Despite the decreased content of sodium ions in tubular urine, against the ground stable decreased clearance and excretory fraction of this electrolyte, the hyponatraemia level did not change.

Hypokaliaemia was found in the group with introduced *Echinacea purpurea,* despite decreased urine concentration and excretion of potassium ions. Under conditions of normal GFR, changes of potassium metabolism indices are caused by the damage of the mechanisms of tubular secretion and reabsorption.

Therefore, maintenance of a low concentration level of sodium and potassium ions in blood plasma might be indicative of the ineffective preventive administration of *Echinacea purpurea,* in case of disturbed ion concentrations due to disorders in ion-regulating kidney function and electrolyte imbalance, when tubules are damaged. It should be noted that in case of toxic nephropathy, as a rule, dysfunction of the nephron proximal portion occurs[16,17]. Thus, decreased proximal sodium ions reabsorption, standardized by glomerular filtration index, in both groups of rats with rapid acetylation type and SCNI, confirms our suggestion concerning development of toxic nephropathy, irrespective of preliminary administration of *Echinacea purpurea.*

It should be noted that in order to determine the relations between acetylation phenotype and efficacy of nephroprotection with pharmacological agents, further studies of kidney functions under conditions of *Echinacea purpurea* tincture administration before SCNI modelling in mature rats with slow acetylation type are needed.

Conclusions

In mature rats with rapid acetylation type, administration of *Echinacea purpurea* tincture before modelling subacute cadmium-nitrate intoxication prevents diuresis changes, which is indicative of maintenance of volume regulating kidney function in case of toxic nephropathy.

The effect of *Echinacea purpurea* is not associated with glomerular filtration rate activation. At the same time, decreased creatinine concentration in blood plasma in rats with subacute cadmium-nitrate damage is indicative of correcting properties of *Echinacea purpurea,* in case retention azotemia is available.

Hyponatraemia and hypokaliaemia indicate the inefficiency of preventive administration of *Echinacea purpurea* in case of naturally determined cadmium-nitrate damages of the mechanisms regulation ion kidney functions and disturbed water-electrolytic balance in mature rats with rapid acetylation type.

The decreased index of proximal sodium ions reabsorption, standardized by glomerular filtration, excludes the protective effect of preventive administration of *Echinacea purpurea* on the tubular nephron portion and is indicative of nephropathy development in rats with rapid acetylation type and subacute cadmium-nitrate intoxication.

Compliance with Ethics Requirements:

„The authors declare no conflict of interest regarding this article"

„The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law."

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