Journal of Advances in Medical and Pharmaceutical Sciences

16(2): 1-16, 2018; Article no.JAMPS.40313
ISSN: 2394-1111

Renal and Metabolic Effects of Goutweed (*Aegopodium podagraria L.*) Extract Compared with Potassium Chloride in Rats Receiving Hydrochlorothiazide

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Authors' contributions

This work was carried out in collaboration between both authors. Authors OVT and SYS designed the study, wrote the protocol and managed the analyses of the study. Author OVT conducted laboratory analysis and collected all data, performed the statistical analysis and wrote the first draft of the manuscript. Author SYS provided the manuscript editing, improvement and completion. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMPS/2018/40313
Editor(s):
(1) Hamdy A. Sliem, Professor, Internal Medicine, Suez Canal University, Egypt and College of Dentistry, Qassim University and EL-Jouf University, Saudi Arabia.
Reviewers:
(1) Salwa Refat El-Zayat, Egypt.
(2) Veeravan Lekskulchai, Srinakharinwirot University, Thailand.
Complete Peer review History: http://www.sciencedomain.org/review-history/23778

Original Research Article

Received 6th January 2018
Accepted 12th March 2018
Published 22nd March 2018

ABSTRACT

Aims: To determine the influence of *Aegopodium podagraria L.* aerial part extract on the function of the kidney and the metabolic processes in rats receiving hydrochlorothiazide (HCTZ, 80 mg/kg).
Study Design: The rats were randomly distributed to five groups: Group I: intact control; Group II: HCTZ; Group III: HCTZ + A. podagraria extract, 100 mg/kg; Group IV: HCTZ + A. podagraria extract, 1 g/kg; Group V: HCTZ + potassium chloride, 60 mg/kg (potassium equivalent).
Place and Duration of Study: Central Scientific-Research Laboratory of National University of Pharmacy, Kharkiv, Ukraine, October 2016 – November 2016.
Methodology: At days 21-22, the renal excretory function was analysed, creatinine, uric acid, urea, protein, sodium and potassium level in urine and plasma were determined, glomerular filtration rate, sodium and water reabsorption, sodium proximal and distal transport were calculated. The signs of

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general toxicity were estimated (relative weights of kidney and liver, plasma marker enzymes). **Results:** No signs of HCTZ general toxicity were seen (and the extract did not induce them when combined with HCTZ), while plasma potassium content was reduced, and the tolerance to some of the renal effects developed. Potassium excretion was maximal in the group receiving GW extract at a higher dose in all experiments, while both doses of the extract, as well as potassium chloride, approximated kalemia to the intact control value. The extract (1 g/kg) and potassium chloride supported natriuresis in water-loading test (due to the decrease in distal sodium transport, $P = .05$) and did not influence on it under the conditions of spontaneous diuresis. The additional favourable properties of the extract are its ability to increase uric acid excretion (at higher dose, $P = .05$) and to decrease plasma urea level (at both doses, $P = .05$).
Conclusion: The results substantiate the principial possibility of goutweed extract combined use with HCTZ.

Keywords: Aegopodium podagraria L.; hydrochlorothiazide; rats; kidney; potassium; sodium.

1. INTRODUCTION

A combined use of the herbal preparations and commonly used drugs attracts much attention nowadays. In such a case there is a possibility to increase the efficacy of the standard therapy as well as its safety. At the same time, there is a risk of unfavourable interactions that substantiate a need in robust research of such combinations [1,2]. Thiazide diuretics including hydrochlorothiazide (HCTZ) are still widely used and continue to be prescribed for the initial therapy of essential hypertension [3]. Many efforts are directed at improving their safety profile. The mechanisms of the metabolic syndrome exacerbation by thiazide diuretics are under intensive research now, and the importance of hypokalemia among these mechanisms has been proven [4] as well as the role of uric acid exchange disorders [5]. Numerous herbal preparations can normalise uric acid metabolism, and they are known as a source of potassium, but these properties have not been investigated widely in the context of thiazides side effects counteraction, although there is evidence of HCTZ efficient combining with herbal preparations. *Hibiscus sabdariffa* L. extract enhances diuretic response to this drug, increases its concentration in plasma and prolongs excretion [6]. *Allium sativum* L. homogenate and *Thea sinensis* L. water extract exert synergistic cardioprotective properties with HCTZ in rats with myocardial damage induced by isoproterenol [7,8], though these studies have not addressed renal function and water-salt metabolism values. The herbal drugs may provide benefits due to the complex composition, in which high potassium content is supplemented with the substances exerting beneficial metabolic effects, e.g. hydroxycinnamic acids and flavonoids.

Our previous results have confirmed the favourable pharmacological activity of goutweed (*Aegopodium podagraria* L., GW) aerial part extract and the tincture, namely antidiabetic, normouricemic, nephro- and hepatoprotective effects [9–12]. These preparations are also characterised by low toxicity level [12]. The extract chemical composition includes hydroxycinnamic acids, flavonoids, components of the polysaccharide-protein complex, micro- and macro elements [11,13]. The extract is of particular interest for the combined use with HCTZ because of the high content of potassium and favourable renal and metabolic effects [10–13]. The previous studies in rats receiving HCTZ and excess fructose have shown that this preparation exerts the hypouricemic effect and a moderate ability to restore sodium reabsorption (which was decreased under the disturbing influence of fructose and HCTZ on the renal concentrating ability) under the conditions of the spontaneous diuresis, as well as antiproteinuric effect and diuresis maintenance under the conditions of hyperuricemia [10,14]. Still, these data were obtained against the background of excess fructose, which itself considerably changes the renal excretory function and thus modulates HCTZ effects. Therefore, there is a need to clarify the interaction between GW extract and HCTZ per se (including the dependence of activity on the dose) and to determine the role of potassium in the extract efficacy. Besides, the rodent diet used in the study [10] did not supply the sufficient intake of sodium. This was seen in the low Na+/K+ ratio in urine as well as low sodium excretion (especially under the conditions of spontaneous diuresis) and possibly contributed to the aldosterone system upregulation which appeared to be unfavourable for the carbohydrate metabolism. That is why it is expedient to study the interaction

between GW extract and HCTZ in animals not undergoing sodium deficiency (which is more physiological and better extrapolated to humans).

Thus, the objective of this research is to determine the influence of *Aegopodium podagraria* L. extract on the function of the kidney and the metabolic processes in rats receiving hydrochlorothiazide.

2. MATERIALS AND METHODS

2.1 Plant Material

The aerial parts of *Aegopodium podagraria L.* were collected from a natural population in Kharkiv region (Ukraine) in June and dried. The extract was obtained by using the standard methods (following the requirements of State Pharmacopoeia of Ukraine) that were described previously [9–11].

2.2 Animal Groups and Treatment

All the experimental protocols were approved by the Bioethics Commission of the National University of Pharmacy and were in accordance with the "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) as well as "Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes."

Female rats with 160 to 220 g body weight were randomly divided into 5 groups (n=5–7 in each group) as follows:

Group I : intact control (IC);
Group II : HCTZ;
Group III : HCTZ + GW extract, 100 mg/kg intragastrically;
Group IV : HCTZ + GW extract, 1 g/kg intragastrically;
Group V : HCTZ + potassium chloride, 60 mg/kg (potassium cation equivalent) intragastrically.

The rats were housed in a well-ventilated animal room at a controlled temperature and relative humidity, on a 12 h light: 12-h dark cycle. Food (standard rodent chow which did not lead to sodium deficiency) and water were supplied ad libitum.

A wide dose range of 3 to 80 mg/kg of HCTZ is used in the experiments as discussed in [15]. At first, a single dose of HCTZ of 20 mg/kg was used for the determination of interactions of GW active components with the moderate dose of HCTZ that was effective in the intact rat [16]. In the subsequent experiments with the prolonged administration, HCTZ was used at a dose of 80 mg/kg, which was chosen according to the data [17] to cause the maximal shifts in metabolism. The doses of the extract were determined in the previous [9–12], the dose of potassium chloride was chosen as an equivalent of potassium, which the animals received with the extract at a dose of 1 g/kg (potassium content was determined in the sample of the extract used in the study, it equalled 5,9%). HCTZ was administered as a suspension (stabilized by polysorbate 80, made ex tempore), GW extract was dissolved in water. The rats of IC group received the same volume of the drinking water. The amount of fluid that the rats in all groups received was similar. The interval between the administration of the preparations and HCTZ equalled 40 min to minimize interaction at the level of absorption. All the groups were treated each day for 3 weeks.

2.3 Biochemical Tests

At day 1, 40 min after the administration of the first dose of HCTZ and the studied preparations, the status of excretory renal function was determined after administration of water loading at a rate of 3% of body weight. Urine was collected for two hours. The rats were previously adapted to the experimental conditions.

At day 16, oral glucose tolerance test was carried out in view of the possible negative influence of HCTZ on the carbohydrate metabolism [4,5,17] and the previously established ambiguous effect of the extract on these metabolic processes under the conditions of HCTZ administration and fructose excess [10]. The rats were fasted for 12 h, than 30% glucose solution was administered intragastrically at a dose of 3.0 g/kg. Blood samples for glucose determination were obtained from a cut at the tip tail at 0, 30, 60 and 120 min. Glucose concentration was measured using the glucose oxidase method, and the total area under the blood glucose curve was calculated using the trapezoidal method.

At days 21–22, the excretory renal function was analyzed against a background of the spontaneous diuresis: after the preparations administration urine was collected in the individual metabolic cages with free access to tap water but without food access. 24-hour diuresis and water intake was recorded and ratio

"excreted/consumed fluid" was estimated. At day 22, 40 min after the preparations administration, the status of excretory renal function was determined as described above. After this, blood samples were drawn by exsanguination from the anesthetized animals. Plasma (the anticoagulant heparin in vitro) was separated immediately by centrifugation. The relative weights of kidney and liver were determined.

The generally accepted routine biochemical methods were applied for blood plasma and urine analysis. Sodium and potassium content in urine and blood plasma was measured using flame photometry method, creatinine – by Jaffe reaction, urea – by the reaction with diacetyl monooxime, uric acid – by the enzymatic method (plasma) and by the reaction with phosphotungstic reagent (urine). Protein concentration in urine was assayed by reaction with sulphosalicylic acid. Total protein concentration in plasma samples was determined by biuret method, albumin level – by the bromcresol green procedure, α-amino nitrogen of low molecular compounds (predominantly aminoacids) – by reaction with ninhydrin after deproteination. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity was determined according to the method of Reitman and Frankel, alkaline phosphatase (ALP) activity – by measurement of the amount of phenol liberated from the hydrolysed substrate. Calcium and inorganic phosphates content in plasma was determined using the reactions with arsenazo III ligand and with molybdic acid, respectively. By the generally accepted formula, creatinine, urea, uric acid, protein, sodium and potassium excretion were calculated, as well as Na^+/K^+ ratio in urine and plasma, urea clearance. Creatinine and sodium concentration in plasma and urine were also used for the estimation of glomerular filtration rate (GFR), relative water reabsorption (R_{H20}), absolute and relative sodium reabsorption, its proximal and distal transport ($RpNa^+$ and $RdNa^+$) [18].

2.4 Chemicals and Reagents

Analytical graded chemicals and reagents were used for this research. HCTZ was sourced from Chinoin Private Co. Ltd. (Hungary). Commercially-available kits from Spainlab Co. Ltd. (Ukraine, kits for uric acid determination in blood plasma) and Filisit-Diagnostika Ltd. SME (Ukraine, the rest of kits listed above) were applied for biochemical assays. Potassium chloride with the grade of purity "chemically pure" corresponding to the requirements of GOST 4234-77 was used (sourced from Alveks Ltd., Ukraine).

2.5 Statistical Analysis

Medians, 25% and 75% percentiles (upper and lower quartiles) were calculated as well as arithmetic means and their standard errors (M±m). The comparison of the central tendencies of independent samples was performed by the Mann-Whitney U-criterion. To determine the relationship between the individual parameters, the Spearman's correlation coefficient of ρ was used.

3. RESULTS AND DISCUSSION

3.1 The Interaction of Goutweed Extract and Potassium Chloride with the Single Dose of Hydrochlorothiazide

The single dose of HCTZ led to the expected increment in sodium excretion and diuresis (Fig. 1). Creatinine excretion was not significantly increased and urea excretion remained unchanged in all of the groups (data not shown). Significant correlation between diuresis and creatinine content in urine appeared in HCTZ-treated rats (–0.90, P = .05 compared with–0.50, P > .05 in the intact animals). The extract blocked the hydrouretic effect of HCTZ at single administration, correlation between diuresis and creatinine content in urine approximated the value of the intact control, being not significant. Its influence on saluresis depended on the dose – the natriuretic effect of HCTZ was maintained against the background of the dose of 1 g/kg (and sodium excretion was intensified even more, than after HCTZ single administration although the differences were not statistically significant, Fig. 1), while the lower dose tended to its reduction. The same dependence on the dose was previously seen in the intact rat [12]. The considerable increment in potassium excretion was seen only in the groups treated with potassium chloride and, especially, the extract at higher dose, it was accompanied with the enhanced natriuresis. Such changes are consistent with the dependence of potassium losses on the diuretic effect of HCTZ [19]. Potassium excretion did not exceed its intake with the extract at a dose of 1 g/kg or potassium chloride, the respective values (medians) equalled 118 and 154 μM per 100 g, while in the group receiving potassium chloride they equalled 77.4 and 154 μM per 100 g. Since 57.5 μM per 100 g were excreted in rats receiving HCTZ per

se, the increment of potassim excretion equalled 60.5 and 19.9 μM per 100 g respectively (the latter was not statistically significant). Thus, the extract led to the more intensive excretion of potassium compared with potassium chloride confirming the involvement of the other biologically active substances into the renal effects of this herbal preparation. Despite the relative decrease in urine volume, the extract at a dose of 1 g/kg did not cause uric acid excretion decline, on the contrary, this value tended to the increase, that is in agreement with the data [12] and may be considered positive. In the other groups this value was not changed.

3.2 The Influence of Hydrochlorothiazide, Goutweed Extract and Potassium Chloride after Chronic Treatment on the Results of the Glucose Tolerance Test

The administration of HCTZ against the background of the standard diet (without excess carbohydrates, lipids or salt) did not lead to the disturbances of the carbohydrate metabolism within the term of the study (Fig. 2). In rats receiving this drug per se, there was only a slight increment of the total area under the blood glucose curve. The extract and potassium chloride tended to reduce glycemia after 60 min. The basal glycemia was not changed in all groups (data not shown) and at the final stage of the experiments there were no significant correlations between blood potassium and glucose (which were seen in the previous studies indirectly indicating aldosterone involvement [10]).

These results confirm that the extract does not cause the negative changes of the carbohydrate metabolism and its unfavourable influence on the latter under the conditions of HCTZ administration and fructose excess [10] can be attributed to the specifity of that model and unphysiological electrolyte intake leading to aldosterone system upregulation (discussed in details in the work [10]). The absence of hypoglycemic action is consistent with the previous data [10,20]. Not influencing the carbohydrate metabolism directly, the extract has another targets of the protective action, such as uric acid metabolism and kidney function.

3.3 The Evaluation of the Possible Toxic Effects of Hydrochlorothiazide, Goutweed Extract and Potassium Chloride after Chronic Administration

Since there was a possibility of the species-specific nephrotoxic effect of HCTZ in rats (it was

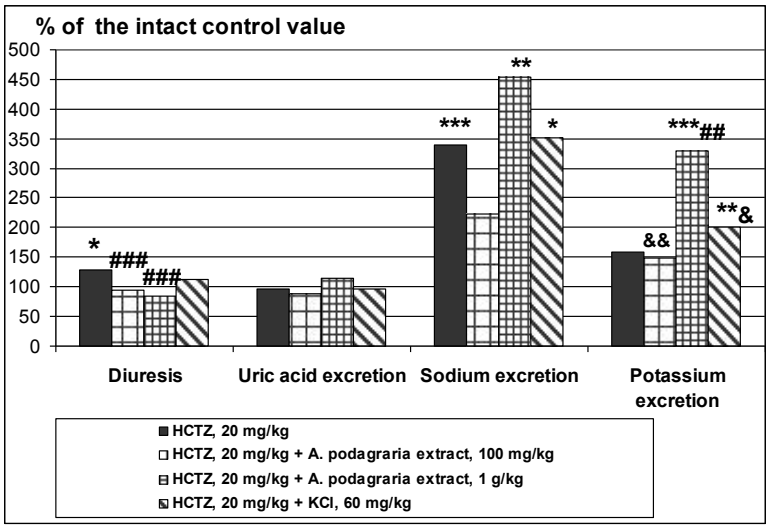

Fig. 1. The influence of hydrochlorothiazide (HCTZ), *Aegopodium podargaria* L. extract and potassium chloride on diuresis, uric acid, sodium and potassium excretion after single administration in the water-loading test in rats

** – P = .05 compared to intact control; ** – P = .02 compared to intact control; *** – P = .01 compared to intact control; ## – P = .02 compared to HCTZ group; ### – P = .01 compared to HCTZ group; & – P = .05 compared to the group receiving the extract at a dose of 1 g/kg; && – P = .02 compared to the group receiving the extract at a dose of 1 g/kg*

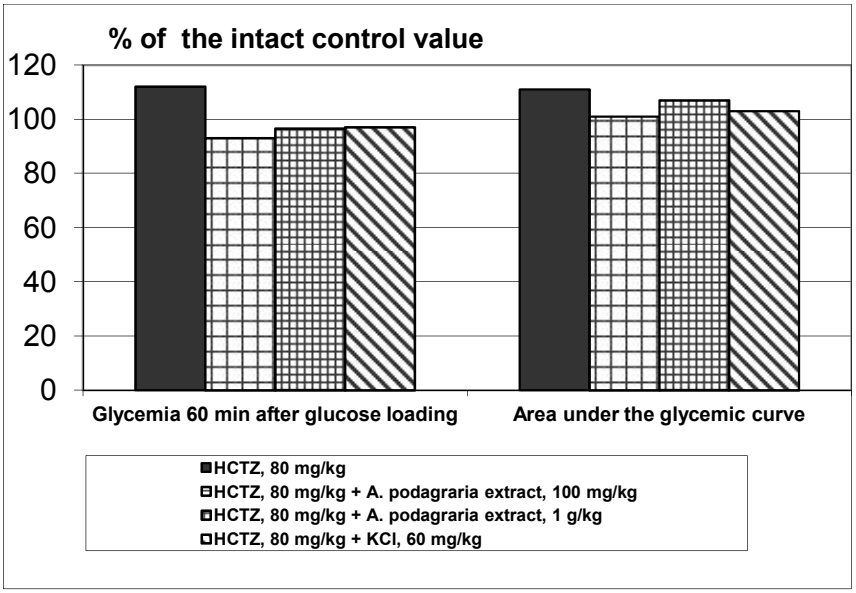

Fig. 2. The influence of hydrochlorothiazide (HCTZ), *Aegopodium podargaria* L. extract and potassium chloride on the results of oral glucose tolerance test in rats

shown in the work [15], though after longer courses of administration) and high doses of HCTZ were used in our study, the values which could indicate renal injury or the general toxic action were determined. The results indicated that the relative weights of kidney and liver were not changed, there was no considerable elevation in the activities of plasma enzymes (data not shown): AST and ALP activity remained unchanged, ALT activity tended to the increase in the animals receving HCTZ per se, while the extract at the high dose and potassium chloride eliminated this tendency. Plasma creatinine concentrations did not differ among the groups and were within the range of 53–64 μM, and plasma urea level was not elevated in animals receiving HCTZ per se. There were no changes in plasma calcium and inorganic phosphate levels, with the exception of a moderate calcium concentration increase in rats receving potassium chloride. Proteinuria was not elevated in any of the groups in water-loading test as well as under the conditions of spontaneous diuresis, in case of the diuresis increment the protein concentration in urine decreased, so its excretion remained unchanged (the values adjusted for creatinine excretion or glomerular filtrate volume were also not changed). Thus, within the period of experiment the signs of toxicity were not observed, that allowed considering the renal effects of the extract as the functional modulation of HCTZ effects rather than the results of the extract nephroprotective action.

3.4 The Effects of Goutweed Extract and Potassium Chloride under the Conditions of Chronic Hydrochlorothiazide Administration

The total protein level in plasma was increased in rats receiving HCTZ alone and with potassium chloride, albumin concentration was elevated slightly (Table 1). Since absolute hyperproteinemia (generally caused by the increased biosynthesis of globulins during the infection process or intoxications [21]) was hardly possible, this increment might be relative, being induced by the intravascular fluid volume decrease, which is possible under the influence of thiazides [19]. The absence of the severe disorders of protein metabolism was also indirectly supported by the stability of the plasma levels of urea and α-amino nitrogen. These changes were also accompanied with the tendency to the increment in plasma sodium content, while potassium level decreased significantly, leading to a change in plasma Na^+ /K^+ ratio (Table 1). Both studied preparations counteracted to hypokalemia development – potassium content had no significant differences from the intact control value. The total protein concentration changes were not statistically significant in the animals receiving the extract at both doses and in rats receiving it at higher dose plasma sodium content approximated to the value of the intact animals. Such properties are quite valuable since potassium supplementation

together with hypovolemia elimination are well known measures of HCTZ side effects elimination [19].

When the the excretory renal function was determined after the prolonged administration of HCTZ (Figs. 3,4), the generally known tolerance phenomenon was seen. Diuresis, creatinine, sodium and potassium excretion as well as GFR, relative water and sodium reabsorption remained unchanged under the influence of HCTZ per se (Fig. 3, Table 2). Unaltered GFR and plasma sodium concentration (Tables 1,2) resulted in the stable filtered load of sodium as well as its absolute reabsorption (data not shown). The distal transport of sodium in rats chronically receiving HCTZ was not decreased, furthermore, a clear tendency to its intensifying was seen (P = 0.1 compared with the intact control value). The absence of the expressed natriuretic effect after chronic administration of diuretics is attributed to the sodium deficit, which initiates compensatory changes in the nephron [22], activation of renin-angiotensin-aldosterone system is possible at that. Besides, the mechanism of action of HCTZ is complex and still under study including the paradoxical antidiuretic effect in diabetes insipidus [23]. It was shown long ago that in the hydrated animals, in contrast to the normal state or dehydratation, the decrease in free water clearance occurs after HCTZ administration [24]. This effect is known to be the secondary reaction to the increased renal sodium excretion with extracellular volume contraction, changes in GFR as well as proximal reabsorption. Moreover, the changes in expression of proteins providing water and sodium reabsorption in the collecting system (in particular, AQP2) were reported [23].

The extract at a dose of 1 g/kg counteracted to the development of tolerance to HCTZ influence on sodium excretion (Fig. 3). It supported natriuresis due to the significant decrease in its distal transport, while the proximal transport remained unchanged (Table 2). After the administration of the extract at the lower dose sodium and potassium excretion as well as reabsorption and ion transport values did not differ from HCTZ-treated group (though potassium excretion was increased significantly compared with the intact control data). Potassium supplementation increased natriuresis to a lesser extent, still the distal transport of this cation was reduced significantly. This indicates the predominant role of potassium among GW components for the realization of natriuretic effect. Nevertheless, potassium excretion was significanly lower when it was given as a solution compared with its administration as a part of the extract. Na+/K+ ratio in urine tended to the decrease in animals receiving the extract at both doses. HCTZ eliminated the negative correlation between diuresis and potassium content in urine inherent in intact rats, while the extract at higher dose restored it (potassium chloride tended to such effect, Table 2).

There are data on the influence of potassium intake on HCTZ effect, still a direct comparison with our results is difficult because these data were not obtained under the conditions of water diuresis, another doses and regimes of HCTZ administration were used, the mechanism of tolerance development was not addressed. In contrast to our results, it was shown that high potassium intake reduces HCTZ natriuretic effect [25] through modulation of sodium reabsorption changes in the distal tubules [26].

Along with potassium, hydroxycinnamic acids are important components of GW extract (the significant intake of these compounds approximating 50 mg/kg is achieved with the higher dose), and their interaction with HCTZ may be expected. Still, despite the abundance of data concerning the metabolic effects of hydroxycinnamic acids (including nephroprotective effects linked to antidiabetic, antioxidative or other activity), there is almost no information about their ability to modulate the activity of the certain sodium transporters. For chlorogenic acid the possibility of potentiation of the phlorizin effect on Na^+/glucose co-transporter (SGLT1) in enterocytes is described [27]. Further research is expected to clarify whether hydroxycinnamic acids exert the modulatory effect on the renal transport systems.

Under the conditions of the spontaneous diuresis, in contrast to the water-loading test, the diuretic effect of HCTZ was evident in all of the groups (Fig. 4). All of the studied preparations increased the efficacy of the excretion of the consumed water (Table 3, HCTZ per se did not cause a statistically significant increase, P = 0.1). The extract at the lower dose decreased water reabsorption resulting in the most significant diuresis increment among the groups, while at the higher dose this effect was not seen. In contrast to water diuresis, the tolerance to HCTZ under these experimental conditions was evident in the absence of natriuretic effect which was not restored by the extract and potassium chloride. These preparations (the extract at higher dose) also intensified the relationship between diuresis and sodium or potassium content indicating the

maintenance of the renal concentrating ability despite HCTZ administration and high potassium intake. The extract at the higher dose caused the most significant increment in potassium excretion (Fig. 4). Na^+/K^+ ratio in urine tended to decrease in both groups receiving the extract (the same changes were seen in water-loading test).

Our results are partially consistent with the data present in the literature. The studies of the renal compensation during chronic HCTZ treatment (though at lower doses and with the different regimen of administration) show that the increase in sodium reabsorption occurs in the collecting ducts, while in the distal segment this process remains suppressed [28] and in such a case the summary excretion of sodium may not be changed. Besides, since the whole 24-hour sample was collected and analysed in our study, it was not possible to determine whether the primary natriuretic reaction developed and was followed by the compensatory retention of sodium.

The presence of hydrouretic effect of HCTZ at chronic administration is attributed to the reduced water reabsorption from the collecting ducts [22]. Thus, HCTZ effects at the level of the collecting duct can be multidirectional depending on the conditions of the kidney functioning: water reabsorption may be inhibited under the conditions of spontaneous diuresis in contrast to water-loading, when ADH secretion is almost completely blocked [29]. The evidence of this was also seen in our study.

The extract at both doses (in contrast to potassium chloride) caused a hypoazotemic effect, significantly reducing urea concentration in plasma (Table 4). Its excretion respectively decreased in the water-loading test (and was higher in animals receiving the extract at lower dose, $P = .086$ compared to the group treated with dose of 1 g/kg) and clearence remained unchanged at both experimental conditions. Thus, it can be assumed that the extract possesses extrarenal mechanisms of the hypoazotemic effect, being able to limit urea synthesis. The similar data were obtained previously in the intact rat, in which the dependence of the effect on dose was also not observed [12].

Uric acid metabolism changes were of special interest. In the water-loading test, a tendency to the increase in uric acid excretion after single administration of the extract (1 g/kg) with HCTZ was seen and the significant uricosuric effect (not connected with the increment of diuresis) developed after their course administration (under the conditions of spontaneous diuresis it did not reach the significant level), while uricemia remained unchanged in all of the groups (Table 4). The correlation between diuresis and uric acid content in urine, which appeared under HCTZ influence was eliminated by the extract and potassium chloride. The role of the decreased uric acid excretion in HCTZ-induced hyperuricemia is long known [30] and the involvement of the certain transport systems (including MRP4 and OAT4) is under intensive study [31]. At the same time, the ability to counteract hyperuricemia through the impact on the renal transporters is proven for herbal drugs containing phenolic compounds [32] as well as such GW component as flavonoid quercetin [33]. For hydroxycinnamic acids, this effect hardly can be expected [34] as well as for trifolin, which predominantly inhibits xanthine oxidase [35].

3.5 Substantiation of the Extract Choice for the In-depth Studies

Proceeding from the previous studies, in which not only the extract but the tincture of Aegopodium podararia L. was studied in rats receiving fructose excess and HCTZ, the question arises about the tincture interaction with HCTZ. We addressed this issue in the separate series of experiments (the groups included intact control, rats receiving HCTZ, and animals receiving this drug and the tincture at a dose of 1 ml/kg used in the previous studies [10,12]) because the respectively low content of potassium [36,37] did not allow to achieve its sufficient intake that was comparable with the extract used. It was shown that the tincture did not lead to the normalization of kalemia. It did not block saluretic and hydrouretic effect of HCTZ single dose (no changes were also seen in urea and uric acid excretion). After its course administration, in the water-loading test it reduced natriuresis due to the intensified sodium transport, the filtered load of which was increased at that (in accordance with higher GFR). Still all of these effects were not present under the conditions of the spontaneous diuresis, while uric acid and urea excretion showed a significant increment in this study. Proteinuria was not changed under both experimental conditions, and no signs of the general toxic action were seen. Hypoglycemic effect of the tincture in the glucose tolerance test did not reach a statistically significant level in rats

receiving HCTZ, this is consistent with the data obtained under conditions of the normal processes of the carbohydrate metabolism – in the intact animals [20], while the active antihyperycemic action developed when the metabolic disturbances were induced by combining of HCTZ with fructose excess [10] as well as on the other models [37].

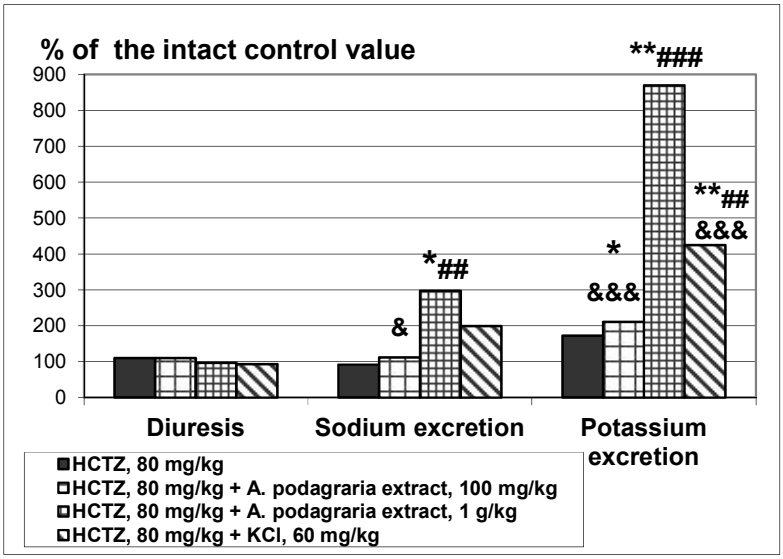

Fig. 3. The influence of hydrochlorothiazide (HCTZ), *Aegopodium podargaria* L. extract on diuresis, sodium and potassium excretion after course administration in the water-loading test

** – P = .05 compared to intact control; ** – P = .02 compared to intact control; ## – P = .02 compared to HCTZ group; ### – P = .01 compared to HCNZ group; & – P = .05 compared to the group receiving the extract at a dose of 1 g/kg; &&& – P = .01 compared to the group receiving the extract at a dose of 1 g/kg*

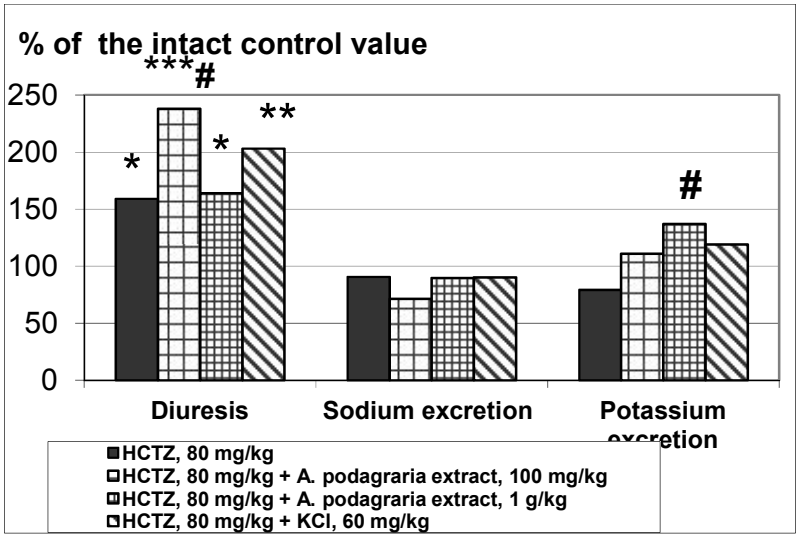

Fig. 4. The influence of hydrochlorothiazide (HCTZ), *Aegopodium podargaria* L. extract and potassium chloride on diuresis, sodium and potassium excretion after course administration under the conditions of the spontaneous diuresis

*Notes. * – P = .05 compared to intact control; ** – P = .02 compared to intact control; *** – P = .01 compared to intact control; # – P = .05 compared to HCTZ group*

Table 1. The influence of hydrochlorothiazide (HCTZ), *Aegopodium podargaria* L. extract (EXTR) and potassium chloride after course administration on the metabolic values in rats; Q50 (Q25–Q75), S.E.M; n=5–7 in each group

	Intact control	**HCTZ, 80 mg/kg**	**HCTZ, 80 mg/kg, + EXTR, 100 mg/kg**	**HCTZ, 80 mg/kg, + EXTR, 1 g/kg**	**HCTZ, 80 mg/kg, + KCl, 60 mg/kg**
Total protein, g/l	**59.8** (58.1–63.2) **59.7±1.99**	**67.5 *** (66.2–68.1) **66.2±2.12**	**68.3** (62.4–70.9) **66.1±2.98**	**65.8** (62.4–66.7) **66.3±2.42**	**65.8 ***** (65.8–67.5) **67.5±1.55**
Albumin, g/l	**30.4** (27.9–33.0) **30.2±1.71**	**32.6** (30.9–34.6) **32.5±1.25**	**34.9** (32.0–35.2) **33.5±1.14**	**31.4** (30.7–33.6) **32.1±1.63**	**32.6** (32.3–33.6) **32.9±0.37**
α-amino nitrogen, g/l	**0.214** (0.210–0.241) **0.232±0.021**	**0.207** (0.199–0.233) **0.214±0.009**	**0.224** (0.199–0.225) **0.208±0.011**	**0.207** (0.192–0.217) **0.203±0.012**	**0.205** (0.204–0.214) **0.220±0.014**
Plasma sodium, mmol/l	**138** (136–146) **141±2.76**	**146** (143–154) **148±3.15**	**147** (143–149) **142±4.75**	**138** (133–142) **138±5.44**	**144** (143–154) **148±3.70**
Plasma potassium, mmol/l	**4.49** (4.35–4.98) **4.71±0.23**	**3.79 *** (3.25–3.99) **3.79±0.36**	**4.86** (4.21–5.05) **4.70±0.28**	**4.60** (3.58–5.94) **4.73±0.67**	**4.61** (4.17–5.13) **4.45±0.52**
Plasma Na^+/K^+ ratio	**31.9** (27.2–33.1) **30.2±1.70**	**38.6 *** (37.9–45.2) **40.9±4.26**	**30.3** (26.2–35.4) **30.9±2.59**	**28.9** (23.9–38.6) **31.2±3.61**	**30.1** (27.9–37.0) **35.4±5.00**
Coefficients of correlation $Na^+ – K^+$	**–0.10** NS	**–0.49** NS	**–0.80** *P*=0.1	**+0.90** *P* = .05	**+0.20** NS

** – P = .05 compared to intact control; *** – P = .01 compared to intact control; NS – P > .05*

Table 2. The influence of hydrochlorothiazide (HCTZ), *Aegopodium podargaria* L. extract (EXTR) and potassium chloride after course administration on the kidney function in the water-loading test in rats; Q50 (Q25–Q75), S.E.M; n=5–7 in each group

	Intact control	**HCTZ, 80 mg/kg**	**HCTZ, 80 mg/kg, + EXTR, 100 mg/kg**	**HCTZ, 80 mg/kg, + EXTR, 1 g/kg**	**HCTZ, 80 mg/kg, + KCl, 60 mg/kg**
GFR, ml/min for 100g	**0.402** (0.396–0.429) **0.412±0.042**	**0.394** (0.362–0.459) **0.399±0.043**	**0.375** (0.293–0.408) **0.377±0.044**	**0.388** (0.290–0.452) **0.377±0.046**	**0.375** (0.325–0.394) **0.351±0.026**
Relative R of H_2O, %	**95.85** (95.42–96.87) **95.77±0.63**	**96.36** (94.42–96.44) **94.91±1.01**	**95.02** (94.71–96.13) **94.96±0.67**	**96.26** (93.90–96.70) **95.21±1.12**	**95.53** (95.39–96.68) **95.59±0.16**
Relative R of Na^+, %	**99.78** (99.69–99.88) **99.72±0.11**	**99.88** (99.70–99.95) **99.72±0.15**	**99.88 &** (99.85–99.89) **99.71±0.18**	**99.24 *#** (99.02–99.42) **99.13±0.20**	**99.43** (99.22–99.76) **99.44±0.16**
Rp of Na^+, mM /2 h per100 g	**6.62** (6.26–6.79) **6.65±0.64**	**6.67** (6.12–7.56) **6.77±0.90**	**5.82** (4.90–6.61) **5.94±0.58**	**6.56** (4.35–7.78) **6.10±0.82**	**6.21** (5.62–6.63) **5.98±0.39**
Rd of Na^+, µM /2 h per100 g	**266** (264–292) **262±22.3**	**334** (290–373) **327±23.9**	**303** (228–324) **283±26.8**	**209 #** (175–218) **212±33.8**	**225 #** (205–262) **239±21.7**
Coefficients of correlation: diuresis – K^+ level	**–1.0**	**–0.36** NS	**+0.11** NS	**–0.89** p<0.05	**–0.54** NS

** – P = .05 compared to intact control; # – P = .05 compared to hydrochlorothiazide group; & – P = .05 compared to the group receiving the extract at a dose of 1 g/kg. NS – p > .05. GFR – glomerular filtration rate, R – reabsorption, Rp of Na^+ – proximal transport of sodium, Rd of Na^+ – distal transport of sodium.*

Table 3. The influence of hydrochlorothiazide (HCTZ), *Aegopodium podargaria* L. extract (EXTR) and potassium chloride after course administration on the kidney function under the conditions of the spontaneous diuresis in rats; Q50 (Q25–Q75), S.E.M; n=5–7 in each group

	Intact control	**HCTZ, 80 mg/kg**	**HCTZ, 80 mg/kg, + EXTR,100 mg/kg**	**HCTZ, 80 mg/kg, + EXTR, 1 g/kg**	**HCTZ, 80 mg/kg, + KCl, 60 mg/kg**
Ratio "excreted/ consumed fluid", %	**43.7** (39.7–72.2) **54.6±9.05**	**71.8** (59.4–97.1) **83.8±15.3**	**84.5 *** (65.2–117) **93.0±15.9**	**85.6 *** (82.1–87.5) **80.9±8.44**	**117 ***&** (113–124) **138±29.1**
GFR, ml/min for 100g	**0.343** (0.322–0.410) **0.365±0.027**	**0.271** (0.247–0.430) **0.322±0.054**	**0.373** (0.261–0.401) **0.336±0.035**	**0.312** (0.260–0.402) **0.344±0.047**	**0.344** (0.269–0.367) **0.332±0.039**
Relative R of H_2O, %	**99.61** (99.55–99.67) **99.56±0.07**	**99.13 *** (98.94–99.43) **99.09±0.20**	**98.93 ***&** (98.55–99.09) **98.85±0.13**	**99.39 *** (99.35–99.39) **99.22±0.17**	**99.12 ***** (98.85–99.26) **98.95±0.21**
Relative R of Na^+, %	**99.55** (99.48–99.77) **99.56±0.12**	**99.46** (99.42–99.69) **99.54±0.07**	**99.57** (99.54–99.81) **99.66±0.07**	**99.59** (99.48–99.61) **99.57±0.04**	**99.55** (99.48–99.77) **99.56±0.12**
Coefficients of correlation: diuresis – K^+ level	**+0.77** *P* = .07	**–0.77** *P* = .07	**–0.25** NS	**–0.90** *P* = .05	**–0.82** *P* = .05
diuresis – Na^+ level	**+0.37** NS	**–0.66** NS	**–0.57** NS	**–0.90** *P* = .05	**–0.86** *P* = .02

** – P = .05 compared to intact control; *** – P = .01 compared to intact control; & – P = .05 compared to the group receiving the extract at a dose of 1 g/kg. NS – P > .05. GFR – glomerular filtration rate, R – reabsorption*

Table 4. The influence of hydrochlorothiazide (HCTZ), *Aegopodium podargaria* L. extract (EXTR) and potassium chloride after course administration on the uric acid and urea metabolism values in rats; Q50 (Q25–Q75), S.E.M; n=5–7

		Intact control	HCTZ, 80 mg/kg	HCTZ, 80 mg/kg, + EXTR, 100 mg/kg	HCTZ, 80 mg/kg, + EXTR, 1 g/kg	HCTZ, 80 mg/kg, + KCl, 60 mg/kg
Plasma uric acid, mmol/l		**0.095** (0.073–0.114) **0.089±0.015**	**0.075** (0.059–0.088) **0.074±0.011**	**0.080** (0.078–0.090) **0.081±0.005**	**0.068** (0.068–0.070) **0.072±0.003**	**0.073** (0.049–0.085) **0.068±0.009**
Plasma urea, mmol/l		**5.691** (5.39–6.47) **5.85±0.32**	**5.34** 5.00–5.99) **5.51±0.26**	**3.97 ** #** 3.97–4.45) **4.21±0.30**	**4.31 * #** (4.30–4.66) **4.37±0.38**	**4.57** (4.38–5.17) **4.95±0.40**
Water-loading test	Uric acid excretion, µmol/100 g for 2 h	**0.60** (0.53–0.78) **0.66±0.07**	**0.60** (0.49–0.69) **0.61±0.05**	**0.61 &** (0.50–0.69) **0.63±0.08**	**0.77 #** (0.75–0.97) **0.85±0.06**	**0.61 &&&** (0.54–0.65) **0.60±0.04**
	Urea excretion, mmol/100 g for 2 h	**0.15** (0.11–0.16) **0.15±0.02**	**0.17** (0.14–0.20) **0.18±0.02**	**0.13 #** 0.12–0.14) **0.13±0.01**	**0.11 *#** (0.10–0.13) **0.11±0.01**	**0.12 #&** (0.12–0.14) **0.13±0.01**
	Coefficients of correlation: diuresis – uric acid level	**–0.10** NS	**–0.75** *P* = .05	**–0.32** NS	**–0.54** NS	**–0.14** NS
	Urea clearance, ml/min for 100g	**0.242** (0.163–0.244) **0.215±0.027**	**0.238** (0.228–0.296) **0.265±0.022**	**0.253** (0.231–0.302) **0.268±0.019**	0.207 (0.196–0.247) 0.218±0.014	**0.259** (0.237–0.362) **0.300±0.038**
Spontaneous diuresis	Uric acid excretion, µmol/100 g for 24 h	**6.70** (5.47–8.01) **6.79±0.71**	**6.22** (5.80–6.63) **6.65±0.79**	**7.25** (5.66–9.17) **7.40±0.88**	**8.11** (7.09–8.99) **7.97±0.83**	**7.91** (5.71–9.36) **7.75±1.00**
	Urea excretion, mmol/100 g for 24 h	**1.07** (1.05–1.60) **1.28±0.16**	**1.27** (1.03–1.39) **1.22±0.13**	**1.14** (0.91–1.39) **1.16±0.11**	**1.20** (1.16–1.44) **1.28±0.17**	**1.10** (0.98–1.37) **1.18±0.12**
	Urea clearance, ml/min for 100g	**0.132** (0.128–0.176) **0.157±0.022**	**0.159** (0.128–0.190) **0.163±0.019**	**0.200** (0.171–0.230) **0.204±0.023**	**0.193** (0.186–0.232) **0.228±0.048**	**0.177** (0.133–0.187) **0.169±0.017**

** – P = .05 compared to intact control; *** – P = .01 compared to intact control; # – P = .05 compared to HCTZ group; & – P = .05 compared to the group receiving the extract (1 g/kg); &&& – P = .01 compared to the group receiving the extract (1 g/kg). NS – P > .05*

4. CONCLUSION

At single administration GW extract, in contrast to potassium chloride, blocked the hydrouretic effect of HCTZ (20 mg/kg) and dose-dependently influenced on its natriuretic effect: it was maintained in animals receiving the extract at a dose of 1 g/kg (as well as potassium chloride) but not at lower dose of 100 mg/kg. Course treatment of rats with HCTZ (80 g/kg) did not result in any changes indicating severe toxicity, while plasma potassium content was reduced, the indirect evidence of intravascular fluid volume decrease was seen (which was eliminated by the extract at higher dose), and the tolerance to some of the renal effects developed. Potassium excretion was maximal in the group receiving GW extract at a higher dose in all experiments, but in all groups of the treated animals, kalemia did not differ significantly from the intact control value. The extract supported natriuresis in water-loading test due to the decrease in the distal transport of sodium, which was also observed in rats treated with potassium chloride, and did not influence on it under the conditions of spontaneous diuresis. It is generally recognized that potassium supplementation eliminates HCTZ side effects many of which arise from the deficiency of this element. It is important that the renal effects of the extract linked to potassium supplementation are favourably combined with its abilities to increase uric acid excretion (at higher dose) and to decrease plasma urea level (at both doses). The results substantiate the principial possibility of goutweed extract combined use with hydrochlorothiazide.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Both authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws ("Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes"). All experiments have been examined and approved by the bioethics committee of the National University of Pharmacy**.**

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/23778