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Effects of *Aegopodium podagraria* Preparations on the Metabolic Disorders Induced in Rats by Excess Fructose Combined WITH Hydrochlorothiazide: The Relationship between Influence on Electrolyte and Carbohydrate Metabolism

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Author's contribution

This whole work was carried out by the author OT.

Original Research Article

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ABSTRACT

Aims: To evaluate the effect of *Aegopodium podagraria* aerial part extract and tincture on electrolyte, glucose and uric acid metabolism in rats receiving excess fructose (10% drinking solution) combined with hydrochlorothiazide (20 mg/kg).
Study Design: Fourty-two male rats were randomly and evenly distributed to seven groups: Group I: intact control; Group II: fructose supplementation; Group III: fructose supplementation + hydrochlorothiazide; Group IV: fructose supplementation + hydrochlorothiazide + *A. podagraria* extract, 1 g/kg; Group V: fructose supplementation + hydrochlorothiazide + *A. podagraria* tincture, 1 ml/kg; Group VI: fructose supplementation +hydrochlorothiazide +*A. podagraria* tincture, 5 ml/kg; Group VII: fructose supplementation + hydrochlorothiazide + reference drug allopurinol, 10 mg/kg. The duration of the treatment was 10 weeks.
Place and Duration of Study: Central Scientific-Research Laboratory of National University of Pharmacy, Kharkiv, Ukraine, between February and 2010 and May 2010.

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Methodology: At week 9, glucose tolerance test was performed. At week 10, the excretory renal function was analyzed, creatinine, uric acid, sodium and potassium level in urine and plasma were determined, glomerular filtration rate, sodium and water reabsorption, Na^+/K^+ ratio of urine and plasma were calculated.
Results: Hydrochlorothiazide against the background of fructose increased sodium excretion and urine Na^+/K^+ ratio (P=.05). The extract and the tincture (5 ml/kg) tended to increase sodium reabsorption while the tincture at the lower dose and allopurinol did not change the renal function. The extract exerted a hypouricemic effect (P=.05). In contrast to the tincture and allopurinol, the extract augmented potassium excretion and reduced its blood content (P=.05). Base on Na^+/K^+ ratios these changes may be associated with unfavourable hyperaldosteronism. The tincture (1 ml/kg) decreased blood glucose level (P=.05).
Conclusion: *A. podagraria* tincture exerts hypoglycemic effect on the model similar to metabolic syndrome. Further study of *A. podagraria* extract dosage regimens is required.

Keywords: Aegopodium podagraria L; goutweed; fructose; hydrochlorothiazide; uric acid; potassium.

1. INTRODUCTION

Plants have represented a valuable source of medicines since time immemorial. Today, according to the World Health Organization data, more than 80% of the world's population rely almost exclusively on traditional medicine for their primary health care needs [1]. There is a growing demand for the verification of herbal drugs phytopharmacological properties.

Aegopodium podagraria L. (goutweed) is a perennial plant of the *Apiaceae* family. It is indigenous to Europe, Siberia, the Caucasus, Kazakhstan and Central Asia mountainous regions and has been naturalized in North America and Australia. The plant is ubiquitous and widely used in traditional medicine for the treatment of gout and related states, rheumatism, kidney and bladder diseases, gastrointestinal tract diseases and as diuretic, anti-inflammatory, and sedative agent. Leaves of the young *A. podagraria* are consumed as vegetable [2–4]. The widespread use of this plant makes it necessary to investigate its possible impact on health.

Several scientific publications concerning phytochemical and pharmacological properties of *A. podagraria* have appeared lately. Hydroxycinnamic acids, flavonoids, coumarins, polyacetylene compounds, essential oil components, micro- and macroelements were identified in it. The compositon of essential oil was established using gas chromatography-flame ionization detector and gas chromatography-mass spectrometry analytical methods. The total and extractable content of mineral and trace elements in *A. podagraria* was determined by atomic absorption spectroscopy. Hydroxycinnamic acids total content was measured using UV spectrophotometry. The capillary electrophoresis method was applied to establishing electrophoretic fingerprints of both the leaves and stems of *A. podagraria* [5–10].

Pharmacological studies have confirmed wide spectrum of *A. podagraria* preparations' activity and low toxicity level. The drugs obtained from its aerial part have a beneficial influence on uric acid metabolism (hypouricemic and uricosuric action) and suppress inflammation [9–11] that confirms its anti-gout properties reflected in the Latin species name. *A. podagraria* extract possesses nephroprotective action in several renal injury models. It

prevents lethality and kidney histostructure changes, normalizes the kidney concentration function and reduces proteinuria and hyperazotemia [11,12]. This preparation also renders hepatoprotective action in the carbon tetrachloride induced hepatitis [13]. *A. podagraria* tincture possesses hypoglycemic effect in intact rats [14] as well as in alloxan-induced diabetic mice [15]. The combination of hypoglycemic, nephroprotective, hepatoprotective, antiinflammatory properties as well as ability to normalize uric acid metabolism can be of great value in metabolic syndrome treatment. Besides, the pharmacological properties of *A. podagraria* to some extent are associated with high potassium content equivalent to 38–83 mg/g (leaves) and 160 mg/g (dry extract, water extraction) according to data [6], 38 mg/g (leaves, 32% of this content is extracted by water) and 77 mg/g (stems, 53% of this content is extracted by water) according to data [5]. It draws additional attention because unfavourable mineral composition of the diet (primarily sodium overabundance and potassium deficiency) is involved in the pathogenesis of metabolic syndrome [16–19]. Severity of metabolic syndrome is also increased by magnesium deficiency [20] and magnesium content in *A. podagraria* raw material is high [6].

Therefore, the objective of this study is to determine *A. podagraria* preparations' efficacy on the model of metabolic disorders that mimic the human metabolic syndrome. This study addresses the influence of *A. podagraria* extract and tincture on glucose metabolism, kidney function and electrolyte homeostasis in rats receiving fructose solution simultaneously with hydrochlorothiazide (HCTZ) administration.

2. MATERIALS AND METHODS

2.1 Plant Material

The aerial parts of *A. podagraria L.* were collected from natural population in Kharkiv region (Ukraine) in June. They were identified by Ass. Prof. Dr. S.I. Stepanova (the Department of Pharmacognosy, National University of Pharmacy, Kharkiv, Ukraine). The herbal raw material was dried at room temperature and powdered using a standard grinding mill. Then the powder was used for the obtaining of the dry extract (double extraction with water followed by evaporation to dryness using rotatory evaporator) and the tincture (double extraction with 70% ethyl alcohol). The technology is standard and corresponds to the requirements of State Pharmacopoeia of Ukraine and was previously described [14,21].

2.2 Animal Groups and Treatment

Noninbred albino rats breeded in the Central Scientific-Research Laboratory of National University of Pharmacy (Ukraine) were used. Male rats with 290 to 310 g body weight were chosen because fructose-induced metabolic disorders in rats have been found to be more pronounced with increasing age [22] and they are androgen-dependent [23].

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 and water were supplied ad libitum.

After one week of acclimation, the rats were randomly divided into 7 groups, each containing 6 animals:

Group I: intact control;
Group II: fructose supplementation;
Group III: fructose supplementation + hydrochlorothiazide;

Group IV: fructose supplementation + hydrochlorothiazide + *A. podagraria* extract, 1 g/kg;
Group V: fructose supplementation + hydrochlorothiazide + *A. podagraria* tincture, 1 ml/kg;
Group VI: fructose supplementation + hydrochlorothiazide + *A. podagraria* tincture, 5 ml/kg;
Group VII: fructose supplementation + hydrochlorothiazide + allopurinol, 10 mg/kg.

The animals of the intact control group consumed tap water while the drinking water was substituted by 10% fructose solution for the other animals. The concentration has been chosen according to data [24,25] because it produces marked pathological changes comparable to the effects of 48-57% fructose in the diet [24]. For the further exacerbation of metabolic disorders HCTZ was additionally administered as proposed in [26]. Under the conditions of metabolic syndrome formation thiazide diuretics prevent hypertension, but increase the severity of disturbances of lipid, carbohydrate and uric acid metabolism and lead to potassium depletion. These changes may accelerate the development of endothelial dysfunction, reduce the availability of NO resulting in further increase of the insulin resistance [26,27]. Since 10 mg/kg is considered to be the minimal dose causing moderate hypokalemia in rats [26] the dose of 20 mg/kg was used in the present study. The drug was administered into the stomach as a suspension (stabilized by polysorbate 80) made ex tempore. The animals of the intact control and fructose only groups (I and II) received distilled water intragastrically.

Allopurinol reduces uricemia and blood pressure, the severity of hypertriglyceridemia, hyperglycemia, insulin resistance against a background of excess fructose combined with HCTZ [26]. So it was used in the present study as a reference drug (Group VII) at 10 mg/kg dose that is recommended in experimental practice [28] and appeared to be effective in previous experiments [11]. In addition, allopurinol was supplied in drinking water at a concentration of 150 mg/l on metabolic syndrome and chronic hyperuricemia models [26,29] providing the quantity of allopurinol consumed similar to the aforesaid dose.

Overcoming of the insulin resistance and hypertension on the chosen model of metabolic disorders is also possible through elimination of HCTZ-induced hypokalemia by replacement of tap water by 1.5–2% solution of potassium chloride [26]. Moreover, it is believed that in the clinic hyperuricemia and hypokalemia are the leading aggravation mechanisms of metabolic syndrome by thiazide diuretics [30,31]. Elimination of these mechanisms is considered as a promising way to increase the efficacy and safety of thiazides [26,27]. These data have attracted our attention due to the ability of *A. podagraria* preparations to counteract oxonate-induced hyperuricemia. *A. podagraria* extract also can compensate for the loss of potassium in certain renal function disorders that has been proven on the model of gentamycine-induced renal injury in rats [11,12]. This drug used in the present study at 1 g/kg dose, exerts hypouricemic and nephroprotective action in rats on different models [11]. The extract was administered to animals of the Group IV intragastrically in the form of aqueous solution.

Normalization of glucose and uric acid metabolism by *A. podagraria* tincture [11,14,15] can also be valuable on metabolic syndrome model. As the tincture effects are dose-dependent and differ in mechanisms [11,14,15], two doses of the tincture 1 and 5 ml/kg were used in the study. Ethyl alcohol was removed before intragastrical administration of the tincture.

A. podagraria preparations and allopurinol were given orally once a day simultaneously with fructose supplementation and HCTZ (for the latter–with 40 min interval to minimize the effect on pharmacokinetics). The animals of the groups I, II, III received distilled water by the similar process. The volume of drugs solutions and suspensions that the rats in all groups

received was similar. The last dose of HCTZ and the studied drugs was given 80 and 40 min before biochemical tests, respectively.

2.3 Biochemical Tests

At week 9, oral glucose tolerance test was carried out. The rats were fasted for 12 h, than 20% glucose solution was administered intragastrically at a dose of 2.0 g/kg. Blood samples for glucose determination were obtained from a cut at the tip tail at 0, 30, 60 and 120 min [22]. Glucose concentration in these samples (as well as in plasma obtained later after anesthesia) was measured using the glucose oxidase method [32]. The total area under the blood glucose curve was calculated using the trapezoidal method [33].

At week 10, the excretory renal function was analyzed against a background of the spontaneous diuresis. The animals were previously adapted to the conditions of the experiment. Then the urine was collected in the individual metabolic cages with free access to tap water or fructose solution but without food access. 24-hour diuresis and water or fructose solution intake was determined and ratio “excreted/consumed fluid” was estimated.

After this the rats were sacrificed under barbiturate-induced anesthesia, blood was obtained by exsanguination and plasma (the anticoagulant heparin in vitro) was separated immediately by centrifugation. The creatinine concentration was measured in urine and plasma using the Jaffe reaction [34], the uric acid concentration – with phosphotungstic reagent [35]. Sodium and potassium levels in urine and plasma were determined using flame photometry method [36].

The 24-hour creatinine, uric acid, sodium and potassium renal excretion was calculated as well as Na^+/K^+ ratio in plasma and urine. Creatinine and sodium concentration in plasma and urine was also used for the estimation of kidney function markers such as glomerular filtration rate (GFR), sodium and water reabsorption (R_{Na+} and R_{H20}, respectively). The following equations were used:

$$GFR = \frac{\text{Urinary creatinine (μmol/l)}}{\text{Plasma creatinine (μmol/l)}} \times \text{Diuresis (ml/min for 100 g)}$$

$$R_{H20} = \frac{\text{GFR (ml/min for 100 g)} - \text{Diuresis (ml/min for 100 g)}}{\text{GFR (ml/min for 100 g)}} \times 100\%$$

$$R_{Na+} = \frac{(\text{GFR (ml/min for 100 g)} \times P_{Na+} - \text{Diuresis (ml/min for 100 g)} \times U_{Na+}}{\text{GFR (ml/min for 100 g)} \times P_{Na+}} \times 100\%$$

PNa+ – plasma sodium level, mmol/l, UNa+ – urinary sodium level, mmol/l.

2.4 Chemicals and Reagents

Analytical graded chemicals and reagents were used for this research. Fructose was obtained from Macrochem Corp. (Ukraine), HCTZ was sourced from Chinoin Private Co. Ltd. (Hungary) and allopurinol from Hexal AG (Germany). Commercially-available kits from Filisit-Diagnostika (Ukraine) were used for biochemical assays.

2.5 Statistical Analysis

All results are expressed as the mean ± standard error of the mean (SEM). The level of significance was defined as p=.05. Statistical differences between groups were analyzed using the Wilcoxon criterion. To determine the relationship between the individual parameters, the Spearman's correlation coefficient of ρ was used.

3. RESULTS

The results showed that 10-week consumption of fructose considerably changed the excretory renal function. In the group of rats receiving fructose, reabsorption of sodium and water decreased with respective augmentation in sodium excretion, urine volume and urinary Na^+/K^+ ratio (Table 1). Effect of HCTZ under such conditions (Group III) was evident only in increase in sodium excretion (p=.05 when compared with group II) due to its reduced reabsorption, with accompanying growth in urinary Na^+/K^+ ratio. Highly statistically significant increase in urine output in comparison with intact control was recorded in all groups of animals (Table 1).

At the same time, drinking activity grew up, so fluid balance ("excreted/consumed fluid" ratio) did not changed substantially. This index was slightly increased against the background of HCTZ (Group III), while *A. podagraria* tincture at a dose of 5 ml/kg and allopurinol showed a tendency towards its reduction. The relationship between diuresis and fluid intake intensified in all groups as evidenced by the correlation coefficient (Table 2).

This interrelation was statistically significant against a background of HCTZ (Group III). Under the influence of *A. podagraria* tincture at a dose of 5 ml/kg diuresis and drinking activity were the lowest among all groups of rats receiving HCTZ, while against the background of *A. podagraria* extract these parameters were the highest. The latter group was different from all others in the absence of a significant correlation between diuresis and creatinine content in urine (Table 2).

There were no statistically significant differences between groups in blood creatinine level, but in all of them this parameter tended to increase when compared with intact control value. Creatinine excretion increased in all experimental groups and the average GFR was not reduced (except group II). It should be noted that GFR in rats receiving HCTZ was highly variable that was evident through SEM (Table 1).

Despite more than fourfold increase in 24-hour sodium excretion against a background of fructose and HCTZ when compared with intact rats value there were no substantial changes in plasma sodium (Table 3). Fructose per se and in combination with HCTZ slightly increased this parameter and only in group of rats receiving allopurinol this augmentation was statistically significant.

Fructose and HCTZ combination also did not cause severe hypokalemia but administration of *A. podagraria* extract lead to the unexpected decrease in plasma potassium (statistically significant differences with data of groups I, V, VI, VII) with respective change in plasma Na^+/K^+ ratio. At the same time, potassium excretion increased by 260% when compared to intact animals value and by 112% when compared to the value of rats receiving fructose and HCTZ (Table 1), differences with all other groups are highly significant). Due to the high kaliuresis the urinary Na^+/K^+ ratio in this group was the lowest.

There was certain dependence on the dose in the influence of *A. podagraria* tincture on electrolyte excretion in rats receiving fructose and HCTZ (Table 1). Against a background of 5 ml/kg dose but not 1 ml/kg, reabsorption of sodium and water was somewhat increased compared with group III and sodium excretion was respectively reduced. The urinary Na^+ /K^+ ratio approximated to the value of rats receiving fructose only and had no statistically significant differences compared with the intact control. Unlike the extract, *A. podagraria* tincture did not substantially affect potassium excretion. There were no significant differences between these parameters in groups II, III, V, VI (but in all of these groups it was augmented compared with intact rats value). Fivefold increase in the tincture dosage lead only to the 18% relative increment in potassium excretion.

The most expressed inter-individual differences of plasma creatinine, GFR, water reabsorption values were registered in the allopurinol group. Allopurinol did not change the natriuretic effect of HCTZ (Table 1). Nevertheless, there was a tendency towards the decrease in potassium excretion so the urinary Na^+ /K^+ ratio reached the highest level among all groups. Unfavourable changes in blood plasma electrolyte content were also recorded against a background of allopurinol (Table 3). Only in this group plasma sodium was significantly higher than in intact rats and plasma potassium was considerably lower compared with the value in animals receiving fructose only.

At week 10, blood uric acid concentration tended to increase under the influence of fructose and, in particular, its combination with HCTZ. The similar values of blood uric acid were seen in animals treated with *A. podagraria* tincture in high dose and allopurinol. In these groups 24-hour uric acid excretion in absolute value was increased compared with the data of intact control, but after recalculation with creatinine excretion taken into account no true augmentation was shown (Table 1). Indeed, the excretion of uric acid in terms of 1 μmol of excreted creatinine is quite similar in all groups (excluding the tendency towards its reduction in rats receiving the lower dose of *A. podagraria* tincture). Unexpected increase in uricemia was observed against a background of the tincture at a dose of 1 ml/kg ($p=.05$ compared with extract-treated group).

There were no changes of the basal glycemia and the results of the glucose tolerance test under the influence of fructose at week 9 (Table 4) that corresponds to the data [22]. HCTZ also did not make worse these parameters. Administration of *A. podagraria* tincture at a dose of 5 ml/kg as well as allopurinol did not lead to any result either. Under the influence of *A. podagraria* extract the highest plasma glucose level (both basal and under the conditions of anesthesia) was recorded, whereas in animals treated with *A. podagraria* tincture at a dose of 1 ml/kg this content appeared to be the lowest among all groups (statistically significant differences even with intact control values). The same phenomena concerned to the areas under the blood glucose curves.

Table 1. Influence of A. podagraria drugs and allopurinol on plasma and kidney function biochemical markers in rats receiving fructose and hydrochlorothiazide

Groups	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
Diuresis, ml/100 g for 24 h	3.01±0.49	7.35±0.87***	9.34±2.18***	10.9±1.29***	9.40±2.05***	7.26±1.01***	9,71±2,07***
Fluid intake, ml/100 g for 24 h	4.06±0.78	9.81±0.98***	12.8±3.62*	13.6±2.02***	12.3±2.57***	10.1±1.46***&	13.6±2.45***
Ratio “excreted/ consumed fluid”, %	71.1±7.16	73.0±5.47	77.9±6.00	75.6±1.52	72.9±3.85	68.5±7.64	68.4±5.90
GFR, ml/min for 100g	0.554±0.092	0.485±0.062	0.623±0.159	0.683±0.109	0.587±0.148	0.645±0.173	0,835±0,325
R H_2O, %	99.60±0.051	98.90±0.16***	98.72±0.38*	98.76±0.23*	98.72±0.34*	98.98±0.23*	98,29±0,64
R Na^+, %	99.98±0.005	99.94±0.008***	99.88±0.036***	99.95±0.015	99.89±0.030***	99.93±0.030*	99,90±0,032*
Sodium excretion, μmol/100 g for 24 h	24.4±4.51	61.1±7.94***	107±18.7***#	71.3±23.6*	112±25.1**	76.2±25.4**	97,8±16,5***
Potassium excretion, μmol/100 g for 24 h	167±16.8	240±35.0	284±31.4**&&&	601±37.4***###	252±16.0***&&&	298±30.1***&&&	212±33,6&&&
Urine Na^+ /K^+ ratio	0.151±0.024	0.267±0.038*	0.375±0.042***#&	0.122±0.040#	0.452±0.112*&^	0.280±0.104	0,522±0,113***#&&& ^
Plasma creatinine, μmol/l	39.2±7.46	49.8±4.75	46.6±8.48	46.0±9.26	47.4±6.90	40.8±9.49	50.7±15.5
Creatinine excretion, μmol/100 g for 24 h	25.1±1.39	32.8±1.30***	33.8±3.12*	38.7±1.61***#	33.2±2.04**&	31.3±2.27*&	31,2±2,96&
Uric acid excretion, μmol/100 g for 24 h	6.00±0.30	7.48±0.63	7.64±0.53*	9.33±0.45***	6.98±0.80&	7.31±0.41*&	6,82±0,66&&
Uric acid excretion, μmol/1 μmol of creatinine	0.240±0.011	0.228±0.015	0.230±0.010	0.239±0.007	0.209±0.017	0.235±0.010	0,224±0,021
Plasma uric acid, μmol/l	0.068±0.013	0.085±0.006&	0.095±0.014&	0.069±0.012	0.111±0.017&	0.085±0.012	0,087±0,014

**GFR: glomerular filtration rate, R H_2O: water reabsorption, R Na^+: sodium reabsorption; Values are expressed as Mean ± S.E.M; n = 6–7.*

Group I: intact control; Group II: fructose supplementation; Group III: fructose supplementation + hydrochlorothiazide;

Group IV: fructose supplementation + hydrochlorothiazide + A. podagraria extract, 1 g/kg;

Group V: fructose supplementation + hydrochlorothiazide + A. podagraria tincture, 1 ml/kg; Group VI: fructose supplementation + hydrochlorothiazide + A. podagraria tincture, 5 ml/kg;

Group VII: fructose supplementation + hydrochlorothiazide + allopurinol, 10 mg/kg;

** – P = .05 when compared to group I, ** – P = .01 when compared to group I, *** – P = .005 when compared to group I;*

 # – P = .05 when compared to group II, ### – P = .005 when compared to group II;

& – P = .05 when compared to group IV, && – P = .01 when compared to group IV, &&& – P = .005 when compared to group IV;

^ – P = .05 when compared to group VI

Table 2. Spearman's coefficients of correlation between the individual biochemical parameters in rats receiving fructose and hydrochlorothiazide treated with *A. podagraria* drugs and allopurinol

Groups	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
Diuresis – fluid intake	+0,37 NS	+0,77 NS	+0,96 (p<0,001)	+0,80 NS	+1,0	+0,60 NS	+0,77 NS
Diuresis – creatinine content in urine	–0,94 (p<0,001)	–0,94 (p<0,001)	–0,96 (p<0,001)	–0,50 NS	–1,0	–0,81 (p<0,02)	–0,83 (p<0,02)
Diuresis – uric acid content in urine	–0,94 (p<0,001)	–0,71 NS	–0,93 (p<0,001)	–0,70 NS	–0,83 (p<0,02)	–0,94 (p<0,001)	–0,77 (p<0,05)
Uric acid content in blood - creatinine content in blood	–0,79 NS	+0,14 NS	+0,54 NS	+0,12 NS	+0,99 (p<0,001)	+0,58 NS	+0,81 (p<0,05)
Uric acid excretion – creatinine content in blood	+0,71 NS	–0,94 (p<0,005)	+0,36 NS	+0,63 NS	–0,61 NS	+0,37 NS	+0,26 NS
Potassium content in blood – basal level of glucose in blood	+0,400 NS	–0,257 NS	–0,700 NS	1,0	+0,500 NS	+0,900 p<0,05	+0,300 NS

NS – p > 0.05

Group I: intact control; Group II: fructose supplementation; Group III: fructose supplementation + hydrochlorothiazide; Group IV: fructose supplementation + hydrochlorothiazide + A. podagraria extract, 1 g/kg; Group V: fructose supplementation + hydrochlorothiazide + A. podagraria tincture, 1 ml/kg; Group VI: fructose supplementation + hydrochlorothiazide + A. podagraria tincture, 5 ml/kg; Group VII: fructose supplementation + hydrochlorothiazide + allopurinol, 10 mg/kg.

Table 3. Influence of *A. podagraria* drugs and allopurinol on plasma electrolytes level in rats receiving fructose and hydrochlorothiazide

Groups	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
Plasma potassium, mmol/l	4.68±0.24	4.45±0.20	4.32±0.19	3.81±0.06*	4.44±0.23$^{\&}$	4.65±0.24$^{\&}$	4.23±0.12$^{\#\&}$
Plasma sodium, mmol/l	141.8±2.69	146.3±4.21	144.3±3.80	142.2±1.48	144.8±1.80	145.8±2.35	148.1±2.06$^{*\&}$
Plasma Na^+/K^+ ratio	30.5±1.32	33.3±1.80	33.6±0.92$^{\&\&}$	37.4±0.71**	33.0±1.58$^{\&}$	31.8±1.99$^{\&}$	35.1±0.67$^{*\#\&}$

Values are expressed as Mean ± S.E.M; n = 5–6.

Group I: intact control; Group II: fructose supplementation; Group III: fructose supplementation + hydrochlorothiazide; Group IV: fructose supplementation + hydrochlorothiazide + A. podagraria extract, 1 g/kg; Group V: fructose supplementation + hydrochlorothiazide + A. podagraria tincture, 1 ml/kg; Group VI: fructose supplementation + hydrochlorothiazide + A. podagraria tincture, 5 ml/kg; Group VII: fructose supplementation + hydrochlorothiazide + allopurinol, 10 mg/kg;

** – P = .05 when compared to group I;*

 # – P = .05 when compared to group II;

& – P = .05 when compared to group IV, && – P = .005 when compared to group IV.

Table 4. Influence of *A. podagraria* drugs and allopurinol on glucose metabolism in rats receiving fructose and hydrochlorothiazide

Groups		Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
Plasma glucose during glucose tolerance test, mmol/l	Basal level	3.99±0.29#	4.01±0.23#	3.63±0.27#	4.58±0.50#	2.88±0.35	4.07±0.33#	4.25±0.30#
	30 min	6.09±0.15##	4.72±0.47	5.35±0.46*	4.77±0.15*	4.50±0.45	5.40±0.47	5.42±0.59
	60 min	5.44±0.81	5.81±0.53	5.26±0.37	6.01±0.97	5.36±0.36	5.72±0.40	5.97±0.20
	120 min	4.39±0.65	4.39±0.43	4.46±0.66	5.53±0.93	3.39±0.45	5.21±0.70#	4.75±0.31
AUC, mmol×min/l		619±51,5	595±44.8	585±30.3	648±56.6#	521±30.6	604±42.3	620±21.8#
Glucose level in plasma obtained after anaesthesia, mmol/l		7.16±0.74	7.91±0.16	7.74±0.49	8.87±0.49##	6.99±0.33	7.72±0.90	7.04±0.38&

AUC: area under the glycemic curve

Values are expressed as Mean ± S.E.M; n = 6–7.

Group I: intact control; Group II: fructose supplementation; Group III: fructose supplementation + hydrochlorothiazide;

Group IV: fructose supplementation + hydrochlorothiazide + A. podagraria extract, 1 g/kg;

Group V: fructose supplementation + hydrochlorothiazide + A. podagraria tincture, 1 ml/kg; Group VI: fructose supplementation + hydrochlorothiazide + A. podagraria tincture, 5 ml/kg;

Group VII: fructose supplementation + hydrochlorothiazide + allopurinol, 10 mg/kg;

** – P = .05 when compared to group I;*

 # – P = .05 when compared to group V, ## – P = .01 when compared to group V;

& – P = .05 when compared to group IV,

4. DISCUSSION

The interest in the mechanisms of metabolic syndrome exacerbation by thiazide diuretics and the ways of their correction has increased lately [26,27,37,38]. Still not much attention has been paid to the possible role of herbal drugs in this context, though their complex composition may stipulate polytropic metabolic effects simultaneously influencing on several pathological pathways. As stated above, *A. podagraria* preparations possess hypoglycemic, nephroprotective, hepatoprotective, hypouricemic and uricosuric, anti-inflammatory properties that are the major preconditions for their efficacy against a background of fructose and thiazides. Furthermore, it has been shown that normalization of blood potassium and uric acid levels can largely prevent the metabolic abnormalities that are associated with thiazides [26] but the activity of herbal drugs in such a context has not been investigated widely. Particularly, there are no data about the relationship between influence of herbal drugs on electrolyte, carbohydrate and purine metabolism. So, the present study focuses the influence of *A. podagraria* extract and tincture on glucose and uric acid metabolism, and electrolyte homeostasis in rats receiving excess fructose combined with hydrochlorothiazide.

According to the results of the current study, severe disorders of uric acid exchange at week 10 were absent. At the same time, the negative correlation between the concentration of uric acid in urine and diuresis present in intact animals disappeared under the influence of fructose (Table 2). This correlation was restored by HCTZ (all groups except IV) nevertheless it was not accompanied by uricemia reduction in animals receiving HCTZ with fructose as well as in those treated with *A. podagraria* tincture. The significant uricemia in animals treated with the tincture at a dose of 1 ml/kg (Table 1) is unexpected, proceeding from our previous results [11], that have confirmed its hypouricemic efficacy relating to extrarenal mechanisms on oxonate-induced model. The absence of hypouricemic action may be attributed both to the methodological aspects of the current study and to the specificity of the metabolic syndrome pathological circuits. Besides, only in the groups of rats treated with *A. podagraria* tincture at a low dose and allopurinol a significant positive correlation between the concentration of uric acid and creatinine in plasma was registered. Interestingly, the same correlation was present in alloxan-induced diabetic mice under the influence of *A. podagraria* tincture at a dose of 1 ml/kg though this model was characterized by hypouricemia and the tincture influence was directed to increase blood uric acid up to normal level [15].

At the same time, *A. podagraria* extract in the current study significantly decreased uricemia (Table 1). At the same dose it was effective on the model of oxonate-induced hyperuricemia combining renal and extrarenal mechanisms of action [11]. In the current study statistically significant relationship between the concentration of uric acid in urine and diuresis disappeared against the background of this drug whereas uric acid excretion was augmented (Table 1) that may indicate the involvement of renal mechanisms into hypouricemic action. Indeed, the extract, in contradistinction to *A. podagraria* tincture, exerts favourable influence on the kidney that is attributed to its protein-polysaccharide complex as well as hydroxycinnamic acids [39]. The content of the latter is higher in the extract than in the tincture [7,11,39]. This assumption is partially confirmed by the data [40]: the herbal drug that contains 4-hydroxycinnamic acid possesses the uricosuric and nephroprotective actions by the regulation of renal organic ion transporters in hyperuricemic mice.

Allopurinol has not exerted hypouricemic effect at week 10 that does not correspond to the results [26] obtained on the similar model at weeks 4, 10, 14. But in the latter study this drug was supplied in drinking water whereas in our work the last dose of allopurinol was

administrated 24 h before blood sampling that might influenced on the results. Data on this xanthine oxidase inhibitor impact on the renal excretion of uric acid are in accordance with our results: reduced excretion in the course administration of allopurinol was not observed [26]. Reungjui S, et al. associate it with a correction of endothelial dysfunction and renal vasoconstriction through hypouricemic action. It is known that allopurinol prevents kidney blood flow reduction under the conditions of hyperuricemia [41]. This phenomenon may also contribute to augmentation of the kidney excretion of uric acid by allopurinol which at first glance seems paradoxical [42].

At the same time, hyperuricemia under the influence of fructose is associated with altered renal excretion of uric acid [43]. The impact on the renal transporters is considered to be a new way of eliminating hyperuricemia and kidney injury in fructose-induced metabolic syndrome. Such action is proved to allopurinol as well as plant-derived flavonoids quercetin and rutin [43]. In addition, differently directed effects on urate transport systems in the kidneys have been proved for HCTZ and allopurinol. So, HCTZ inhibits MRP4 – the only known carrier that provides transport of urate through the apical membrane of proximal tubular cells. Taking into account pharmacokinetics, the realization of this effect in vivo is quite possible. Allopurinol and its active metabolite oxypurinol exhibit the opposite effect and enhance transport of urate by the above-named transporter that may be involved into the mechanism of hypouricemic action [44].

According to the results of the present study, fructose excess per se significantly changed the excretory renal function. Nevertheless, hyperfiltration and increase in blood creatinine were not evident (Table 1) in contrast to the data in the literature [41,45]. The renal dysfunction in fructose-induced metabolic syndrome is also associated with alterations of transport systems. Thus, the 4-week consumption of fructose increased the abundance of the sodium-phosphate co-transporter, whereas it reduced the bumetanide-sensitive Na^+-K^+-$2Cl^-$- cotransporter and aquaporin-2 [23]. Reabsorption disorders were also found in the present study. At the same time, in animals treated with *A. podagraria* extract and with the tincture at a dose of 5 ml/kg there was a tendency towards sodium reabsorption maintenance with respective decrease in its excretion compared with group receiving fructose and HCTZ. The latter caused statistically significant augmentation of sodium excretion only in rats untreated with *A. podagraria* preparations and allopurinol (Group III) so these drugs to some extent counteracted this augmentation. Also there were no statistically significant differences in the excretory renal function markers (such as diuresis, “excreted/consumed fluid” ratio, GFR, sodium and water reabsorption, sodium excretion, Table 1) between these groups (IV–VII) and compared with Group II as well as with Group III.

The results also have indicated an unexpected phenomenon: the reduction of blood potassium against a background of *A. podagraria* extract that is extraordinarily rich in this element [5,6]. Plasma Na^+ /K^+ ratio was respectively augmented (Table 3). In contrast to these results, there was no decrease in blood potassium in all previous experiments [11,12]. In the present study the extract substantially increased potassium excretion with minor changes in sodium excretion that logically lead to the urinary Na^+ /K^+ ratio reduction (Table 1). The latter under the conditions of fructose excess and HCTZ administration with the extract was lower than the value of intact rats and had statistically significant differences from all other groups (except for group VI with high inter-individual variability of this value). As such changes in plasma and urinary Na^+ /K^+ ratio are usually considered to be an reliable sign of increase in distal tubules mineralocorticoid control [46], aldosterone involvement in the extract-induced hypokalemia is possible.

Similar data are available in the literature: diuretic and natriuretic effects of herbal hypoazotemic drug Lespeflan were accompanied by increased level of aldosterone in the blood [47]. Decoction of *Ficus racemosa L.* roots at 250–1000 mg/kg doses, the highest one is similar to the dose of *A. podagraria* extract used in our studies, reduced the urinary Na^+ /K^+ ratio (sodium excretion and diuresis were decreased) [48].

Our earlier results [11] also indicated the possible enhancement of aldosterone influence by *A. podagraria* extract, namely the reduction in the urinary Na^+ /K^+ ratio in intact animals both in spontaneous diuresis and in water-loading test. We have regarded these changes as a counteraction to homeostasis disturbance: the primary rapid natriuretic effect of the extract may cause reactions aimed at diuresis stabilization and protection of the body from dehydration [11,16].

Increase in aldosterone blood level is the physiological consequence of excessive potassium intake. Potassium, that is present in high quantity in many herbal drugs, including *A. podagraria*, participates in the realization of their diuretic activity and other pharmacological effects. It is this specifity of plants mineral composition that has become an evolutionary prerequisite for the formation of blood potassium level control through aldosterone [49].

Augmented aldosterone level as the result of high potassium intake was also shown in Dahl salt-sensitive rats, and excessive dietary KCl as well as limited one caused hypertension at that [50]. Diet high in potassium counteracted the natriuretic effect of HCTZ in Long Evans rats [51]. Hyperaldosteronism was observed with long-term administration of HCTZ and was associated with subtle renal injury [52]. At the same time, in the present study the possible hyperaldosteronism in animals receiving *A. podagraria* extract, fructose and HCTZ (Group 4) was not accompanied by a decrease in creatinine clearance and augmentation of its blood level (Table 1). This data is consistent with the proven nephroprotective action of the extract [11].

Thus, the aforesaid data suggest a possible relationship between hypokalemia in animals receiving *A. podagraria* extract combined with fructose and HCTZ and aldosterone system. At the same time, as is generally known, hyperaldosteronism intensifies thiazide-induced hypokalemia [31] and the result was seen in the fall of blood potassium in the animals of the group IV.

Hypokalemia, in turn, is considered as a probable mechanism of glucose metabolism disorders caused by thiazide diuretics [30,31]. It is known that potassium exchange is an important link between carbohydrate metabolism and renin-angiotensin-aldosterone system, and numerous feedbacks are present in this system [19]. Increased intake of potassium counteracts the negative influence of thiazides on carbohydrate metabolism [26,30]. But the expected favorable effect of *A. podagraria* extract as a source of potassium on the glucose metabolism has not been proven in the present study. In contrast, the maximal levels of blood glucose as well as the area under the blood glucose curve were recorded in animals receiving this drug (Table 4). Perhaps the reason is in the above-named changes in potassium homeostasis with possible chronic increase in blood aldosterone. Positive correlation between blood potassium and glucose under the influence of extract (Table 2) may indirectly indicate this aspect. It has been shown in vivo and in vitro that glucose intolerance under the conditions of relative aldosterone excess is connected with decreased glucose-stimulated insulin secretion independent of mineralocorticoid receptors [53]. Moreover, aldosterone stimulates gene expression of gluconeogenic enzymes through the

glucocorticoid receptors, thus affecting the inhibitory effect of insulin on hepatic gluconeogenesis [54].

In contrast to the extract, *A. podagraria* tincture at a dose of 1 ml/kg has exerted a hypoglycemic effect. Blood glucose level in this group differed significantly from all other groups including intact control (Table 4). This effect was present even under the conditions of anesthesia. The area under the blood glucose curve was also decreased by *A. podagraria* tincture while the blood glucose concentration after 30 min and after 120 min was minimal among all groups. Increasing of the tincture dose to 5 ml/kg lead to the loss of hypoglycemic activity and correlation between blood potassium and glucose in this group approximated the value of group receiving *A. podagraria* extract (Table 2). In contrast to these results, hypoglycemic action of the tincture in the intact rats has been shown at a dose of 5 ml/kg [14]. In alloxan-induced diabetic mice both doses of *A. podagraria* tincture reduced glycemia, but taking into account the influence on the survival rate and all biochemical parameters, the lower dose appeared to be more effective [15]. Such different dose-effect relationship with higher dose needed for the effect realization under normal conditions compared with the dose effective in carbohydrate metabolism impairment may be regarded as valuable. Further studies are required to characterize the mechanisms of hypoglycemic action and their relationship with other metabolic effects as well as chemical composition. The tincture components are proven to be not responsible for *A. podagraria* nephroprotective properties [39] still they have appeared to be significative for carbohydrate metabolism normalization. The study of the tincture influence on lipid and protein metabolism seems to be promising.

As to *A. podagraria* extract, in further studies it is expedient to use the lower doses for the establishment of the dependence of activity on the dose. Administration of the extract once a day (that was used in the current study in order to achieve strict standardization) appeared to be unfavourable because of sharp potassium loading. So another dosing regimen should be applied in future. In the papers cited above [16,26] potassium compounds were dissolved in tap water ensuring auspicious pharmacokinetics. Moreover, in the current study standard rodent diet was used while the diet with increased sodium content may approximate the model to the real situation in humans. It is known that fructose-induced pathological changes are less expressed when consuming a diet with a normal (1:3) ratio of sodium and potassium in contrast to high sodium diet [55] as well as under the conditions of chloride-free diet [56].

4. CONCLUSION

The results of this study partly confirm *A. podagraria* favourable metabolic properties known in traditional medicine. On the model similar to metabolic syndrome *A. podagraria* tincture (1 ml/kg) exerts hypoglycemic effect and *A. podagraria* extract (1 g/kg) shows hypouricemic properties. At the same time, *A. podagraria* extract, that is rich in potassium, at 1 g/kg dose leads to the changes that may be associated with hyperaldosteronism. Further study of A. *podagraria* extract dosage regimens is required.

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ETHICAL APPROVAL

The "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the bioethics committee of the National University of Pharmacy.

COMPETING INTERESTS

The author has declared that no competing interests exist.

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