Original Research Article

Behavioural Reactions of Random-Bred Mice under the Influence of Hypouricemia and Aegopodium podagraria L. Preparations

ABSTRACT

Aims: Aegopodium podagraria L. (goutweed) preparations normalize uric acid metabolism and exert organoprotective effects. Still their efficacy was not determined in combined use with therapeutic doses of allopurinol. This study adressed the changes of uric acid metabolism and CNS in mice undergoing hyperuricemia correction with allopurinol combined with goutweed extract and tincture.

Study design: The mice were randomly distributed to five groups: Group I: intact control; Group II: control for manipulations and hypouricemia (allopurinol, 2.5 mg/kg); Group III: potassium oxonate (PO), 250 mg/kg + allopurinol, 10 mg/kg; Group IV: PO, 250 mg/kg + allopurinol, 10 mg/kg + extract, 1 g/kg; Group V: PO, 250 mg/kg + allopurinol, 10 mg/kg + tincture, 1 ml/kg.

Place and Duration of Study: Central Scientific-Research Laboratory, National University of Pharmacy, Kharkiv, Department of Biochemistry, Kharkiv National Medical University, June 2017 – September 2017.

Methodology: Beginning from the 15th day psychopharmacological tests were carried out. At day 21, xanthine oxidase activity in the liver and kidney, uricase activity in the liver, uric acid level in blood and brain, GABA, serotonin, aspartic and glutamic acids concentrations in the brain were determined.

Results: The extract and the tincture did not counteract the influence of allopurinol on xanthine oxidase, liver uric acid level was decreased and uricemia slightly elevated (especially by the tincture) allowing to suggest the changes of uric acid transport. Such changes also were possible in brain resulting in the increased uric acid level in the animals receiving combinations with goutweed or allopurinol per se, and the decrease in GABA, the increment in aspartic and glutamic acids were seen in these groups. The extract and especially the tincture decreased brain serotonin level which was elevated by PO and allopurinol. Allopurinol per se mildly reduced locomotion activity, the same changes were induced by combinations with goutweed preparations. Allopurinol and PO increased the duration of stay in the open arms of the elevated plus maze that was eliminated by the tincture, which also normalized the number of mice immediately visiting the open arm. The extract and the tincture decreased depressivity level in the tail suspension test. The tincture restored physical endurance in the weight-loading forced swimming test.

Keywords: uric acid, potassium oxonate, allopurinol, central nervous system, Aegopodium podagraria L., mice

1. INTRODUCTION

Hyperuricemia is a widely discussed risk factor of cardiovascular diseases, diabetes mellitus and kidney injury [1–3]. At the same time its significance is not limited to these aspects, and the relationship between purine metabolism and CNS activity is under research. Evolutionary advantages of the loss of uricase and respectively high uricemia are supposed, being important for the appearance of the intellectually developed primates well as, in later periods of human development, for the formation of high achievements up to genius development [4–5] or, at least for everyday activities [6]. In this context, disadvantageous effects of the low uric acid level in blood are reported, and the treatment of hyperuricemia should not result in the excessive decrease in this value [3].

Herbal preparations are promising for hyperuricemia counteraction through the different mechanisms (hypouricemic action, uricosuric effect or the influence on free radical processes associated with urate synthesis as well as for other processes pathogenetically important in hyperuricemia) [7] Also a great body of evidence exists about the favourable psychotropic effects of the herbal drugs [8], and multidirectional effects are possible because of their complex composition. Still the relationship

between antihyperuricemic and psychotropic activity of the herbal preparations is not completely elucidated in the data in the literature.

Our efforts are focused on the pharmacological studies of the preparations obtained from the aerial part of goutweed (GW, Aegopodium podagraria L., Apiaceae). It is a perennial plant widely used in traditional medicine and consumed as vegetable. The plant is ubiquitous and the raw material of the goutweed aerial part is available for drug manufacturing at respectively low cost. The Latin species name was given to the plant by Linnaeus in accordance with its use in gout; and pharmacological research has confirmed this approach: hypouricemic and uricosuric action of water extract and tincture obtained from Aegopodium podagraria L. aerial part was described (together with the beneficial nephroprotective, hepatoprotective, antihyperglycaemic properties) [9–11]. In normouricemic animals, these preparations do not cause a significant shifts in the behavioural reactions, and moderate favourable influence of anxiety and depression signs was seen, being dose-dependent and sex specific [12]. Still there is no data about the influence of Aegopodium podagraria L. preparations on the behavioral reactions of the animals with the changes in purine metabolism, including those induced by allopurinol (ALL). The latter is of special importance because of the interest to the herbal drugs use in combined therapy of hyperuricemia. Besides, ALL possesses a certain influence on the CNS [13]. Proceeding from the low uric acid level inherent in the intact rodents, it is expedient to use ALL on the model of hyperuricemia. The preliminary results confirming the absence of the blockade of ALL effects against the background of GW tincture as well as the absence of toxicity increase have been obtained recently [14].

Therefore, the aim of this study was to determine the effects of Aegopodium podagraria L. extract and tincture in mice receiving allopurinol, namely the changes in depression and anxiety signs, physical endurance, locomotor activity and exploratory behaviour as well as state of purine metabolism (uricemia, xanthine oxidase and uricase activity).

2. MATERIAL AND METHODS

2.1 Plant material

The aerial parts of Aegopodium podagraria L. were collected from the natural population in Kharkiv region (Ukraine) in June. The herbal raw material was dried at room temperature and powdered using a standard grinding mill to obtain the powder with the mean particle size of approximately 2 mm. The powder was twice extracted with water at 90ºC. The plant material and solvent were taken in 1:10 ratio, the solvent volume was increased according to the swelling index. The extract was filtered under vacuum conditions and concentrated using a rotary evaporator, and a dry solid was obtained (residual water content equalled 5%), corresponding to an average yield of 25%. Goutweed dry extract is a brown powder with a characteristic pleasant odour, sour-bitter to taste.

Goutweed tincture was prepared by double extraction with 70% ethyl alcohol. The plant material and solvent were taken in 1:5 ratio, the solvent volume was increased according to the swelling index. The solvent was divided into two parts. The plant material was macerated in 2/3 solvent at room temperature for five days being periodically shaked and stirred. The mixture was filtered under vacuum conditions and maceration process was repeated under the same conditions with the rest of the solvent. The obtained liquids were combined into one, kept for two days at 4ºC, filtered and brought to the calculated volume with the solvent. Goutweed tincture is dark green liquid with a characteristic odour. The standard technology of Aegopodium podagraria L. dry extract and tincture obtaining was in accordance with the requirements of State Pharmacopoeia of Ukraine and was described previously [9,10].

2.2 Chemicals and reagents

Analytical graded chemicals and reagents were used for this research. Potassium oxonate was sourced from SigmaAldrich (St. Louis, MO, USA), ALL – from Hexal AG (Germany). Commercially-available kits from Spainlab Co. Ltd. and Filisit-Diagnostika Ltd. SME (Ukraine) were used for the measurement of uric acid level in blood plasma and brain tissue, respectively. Other chemicals used were of analytical grade.

2.3 Animal groups and treatment

Adult random-bred male mice (body weight 22–26 grams) were obtained from the Central Scientific-Research Laboratory of National University of Pharmacy. All the experimental protocols were approved and in accordance with "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". The mice were housed in a well-ventilated animal room under a controlled temperature and relative humidity. Food and water were supplied ad libitum.

After acclimation, mice were randomly assigned to 5 groups:

Group I: intact control without any manipulations (IC, n=8);

Group II: control for manipulations (M) and hypouricemia (intragastrical administration + allopurinol, 2.5 mg/kg, M +ALL, n=7);

Group III: potassium oxonate, 250 mg/kg + allopurinol, 10 mg/kg, (ALL+PO, n=7);

Group IV: potassium oxonate, 250 mg/kg + allopurinol, 10 mg/kg + GW extract, 1 g/kg (ALL+PO+EXTR, n=7);

Group V: potassium oxonate, 250 mg/kg + allopurinol, 10 mg/kg + GW tincture, 1 ml/kg (ALL+PO+TINCT, n=7);

In addition to the intact control, the control group was used, undergoing intragastric administrations and receiving ALL at the minimally effective dose of 2.5 mg/kg [15]. The groups receiving ALL or PO per se were not used, since we addressed the changes of CNS against the background of these agents in the previous study [13] and in the current work we aimed just at the effect of GW preparations against the background of ALL use in hyperuricemia which is directed to the substantiation of these herbal drugs use in the combined treatment of gout (and the use of the additional groups complicate the scheme of the study and prolong the psychopharmacological tests making them less synchronous).

ALL was administered at a dose of 10 mg/kg intragastrically [10,13]. For the increase in uricemia, potassium oxonate (PO) was given (it is widely used in psychopharmacological studies despite the relatively short effect [16]), and effective increase in uricemia as well as changes of behavioral reactions were reached in our previous studies after its intragastric administration at a dose of 250 mg/kg [13] which was used in the current study. GW extract and tincture were used at doses 1 g/kg and 1 ml/kg respectively which are most promising for purine metabolism disorders correction [9,10,14].

All studied drugs and chemicals were administered intragastrically twice a day (in the following order: PO, ALL, goutweed preparations) in 6–8-hour interval. The mice of the control groups received intragastrically drinking water by the similar scheme. The amount of fluid that the mice in all groups received was similar.

Beginning from the 15th day psychopharmacological tests were carried out. The same mice were used for all the tests to make correlation analysis of the results possible. The interval between drug administration and the beginning of the study was 1 h for each mouse. All behavioural tests were conducted from 11 a.m. up to 6 p.m.

The procedures were conducted in a sound attenuated room. The animals were transported from the housing room to the testing area in their own cages and were allowed to adapt to the new environment for 1 h before testing. The test equipment was thoroughly cleaned using alcohol solution followed by drying before each mouse was tested.

2.4 Behavioural tests

2.4.1 Combined open field

The mice were observed in an open field arena, 22 × 22 × 11 (L × W × H) with floor divided into 16 squares with 16 holes (1.5 cm diameter). After 3 min in a dark cage, the mouse was placed on one of the peripheral squares. During a 3-min test period, the following measures were taken: the number of squares crossed, the number of times the animal reared and made exploratory nose-pokes, the number of fecal boli, urinations, and grooming acts.

2.4.2 Elevated plus maze

The elevated plus maze apparatus [17] was made of plastic, glass and wood. It consisted of two brightly-lit open arms (glass surface), 10 × 50 (L × W), two opposed enclosed arms 10 × 10 × 50 (L × W × H), open to the top. The maze was elevated to a height of 1 m. After a 5-min period in a dark cage, the mouse was placed in the center of the maze, facing one of the open arms. The test period lasted for a 5 min. Traditional anxiety measures were taken, such as the number of entries into the enclosed arms and into the open arms, and time spent in the different compartments. Besides, the latency of entry into the open arm and into the enclosed arm, the number of times the maze center was crossed were registered as the widely used additional characteristics of anxiety as described in [12]. Also the number of fecal boli and urinations was registered.

2.4.3 Tail suspension test

The mice were suspended on the support by adhesive tape placed approximately 1 cm from the tip of the tail [17]. The distance from the mouse's nose to the table top was 10 cm. The duration of immobility was recorded for a period of 6 min. The number of fecal boli was also taken into account.

2.4.4 Weight-loaded forced swimming test

Weight-loaded forced swimming test was conducted after a course of GW preparations administration (10 doses). The metal load (10% of body weight) was fixed on the tail root of each mice and the animals were placed individually into the pool with water at 22–23°C. The pool was filled with 60 cm water, pool ledges equalled 15 cm over the water level, not allowing the rest of the animal on them. Swimming time to exhaustion by the criterion of head dip under water without coming to the water surface for 10 seconds was recorded [18] with modifications.

2.4.5 Extrapolation escape task

Cognitive functions were assessed by extrapolation escape task, registering the latency of escape (avoidance through diving) of the animal placed to the cylinder, the edge of which is under water, as described in [19]. The test period was limited to 120 seconds, and the number of animals that solved the task was recorded.

2.5 Determination of the biochemical values

After all the psychopharmacological tests were accomplished, the animals were taken out of the experiment under barbiturate-induced anesthesia (60 min after the last drug administration). The mice were fasted for 12 h before taking final blood samples but they were allowed free access to tap water. Blood was obtained by exsanguination and plasma (the anticoagulant heparin in vitro) was separated immediately by centrifugation. The level of uric acid in the plasma was measured by the uricase method. The concentration of uric acid and xanthine oxidase (XO) activity in the liver and kidney were determined (spectrophotometric determination of the synthetized uric acid against the background of uricase blockade), in the liver uricase activity was additionally measured [20,21], the methods and modifications were described previously in details [11].

The levels of GABA, aspartic acid, glutamic acid were determined in brain tissue using the method of high voltage electrophoresis and uric acid level – by using the standard kit as stated above. Serotonin concentration was measured by the procedure derived from the solvent extraction technique and o-phtaldialdehyde methods for the development of fluorophores. The increase in sensitivity was accomplished by volume reduction accompanied with changes of reagent concentration [22].

2.6 Statistical analysis

Taking into consideration the absence of normal distribution for most of data, medians, 25% and 75% percentiles (upper and lower quartiles) were calculated. Traditionally used means ± standard errors of the mean (SEM) were also shown (M±m). Statistical differences between groups were analysed using the Mann-Whitney U test (taking into account a problematical character of multiple comparisons in pharmacology and toxicology [23]) and the Fisher angular transformation. The level of significance was defined as $p<0.05$. To determine the relationship between the individual parameters, the Spearman's correlation coefficient of ρ was used.

3. RESULTS AND DISCUSSION

3.1 The results of the biochemical studies

A significant uricase inhibition was achieved in all animals receiving PO (and there were no differences between these groups) confirming the bioavailability of PO and the absence of GW preparations direct interactions with it (Table 1). ALL administration led to an expected decrease in uricemia in all of the treated groups due to XO inhibition (a tendency in mice receiving this drug in a minimal dose and a significant blockade with the results below detection limit after administration of the dose of 10 mg/kg). In the previous studies [10,11] ALL at the same dose inhibited XO to a lesser extent, and the reason for this may be the prolonged use of this drug with an administration twice a day. Uricemia in all of the groups receiving ALL and PO was decreased almost threefold compared with IC value, in mice treated with GW preparations, especially the tincture, this value was higher than in PO+ALL group. Since liver uric acid level in GW-treated mice was significantly lower (in both groups $P = .08$ compared with the value of PO+ALL group) and in PO+ALL group it did not differ significantly from both control groups, the influence on the transport systems can be supposed. Liver XO activity in all of these three groups was reduced and it is consistent with the previous data confirming that GW tincture does not interfere with ALL main effect (at high doses of ALL) [14], and the similar level of XO inhibition and uric acid concentration was seen in kidney of these mice. We have not determined the excretory renal function in this study, still it is known that GW preparations can support uric acid excretion [10,11]. The data concerning the influence of the herbal biologically active substances on the certain uric acid transporters are scarce, still there is evidence that flavonoids (including quercetin and its derivatives which are present in GW) are able to influence the transporters pathogenetically important in uricemia [24,25].

It is more difficult to explain the increase in brain uric acid level in mice receiving ALL at a low dose (M+ALL group, Tab. 2). It is known that in hypouricemia as well as in hyperuricemia the level of uric acid in blood serum correlates closely with its level in brain [26]. At the same time, the systems providing uric acid transport (including transcellular transport) within the blood-brain barrier are under intensive research now.

Table 1. The effect of allopurinol and *Aegopodium podagraria* L. preparations on the purine metabolism values of potassium oxonate-treated mice, **Q_{50} (Q_{25}–Q_{75});** M±m, n=6–8

	IC	M + ALL	PO + ALL	PO + ALL + EXTR	PO + ALL + TINCT
Uricemia, mMol/l	**0.093 (0.079–0.100)** 0.094±0.008	**0.070 *** **(0.058–0.073)** 0.066±0.009	**0.030 ***&& **(0.026–0.033)** 0.029±0.002	**0.040 ***&& **(0.032–0.044)** 0.038±0.004	**0.035 ***& # **(0.033–0.041)** 0.038±0.003
Uric acid in liver, µM/g tissue	**0.38 (0.31–0.38)** 0.35±0.02	**0.35 (0.32–0.38)** 0.35±0.03	**0.35 (0.29–0.36)** 0.33±0.02	**0.27 *& (0.23–0.30)** 0.25±0.03	**0.24 * (0.16–0.32)** 0.24±0.04
Liver XO activity, ncat/g tissue	**0.39 (0.34–0.41)** 0.39±0.06	**0.17 (0.15–0.33)** 0.23±0.06	**0** (BDL)	**0** (BDL)	**0** (BDL)
Liver uricase activity, ncat/g tissue	**0.051 (0.047–0.077)** 0.066±0.019	**0.037 (0.019–0.057)** 0.039±0.013	**0.016 * (0–0.020)** 0.013±0.005	**0 (0–0.037)** 0.017±0.011	**0.006 **& (0–0.017)** 0.012±0.006
Uric acid in kidney, µM/g tissue	**0.21 (0.14–0.24)** 0.20±0.03	**0.18 (0.13–0.21)** 0.17±0.02	**0.08 **& (0.06–0.10)** 0.09±0.02	**0.10 **& (0.06–0.11)** 0.09±0.02	**0.10 **& (0.08–0.11)** 0.10±0.01
Kidney XO activity, ncat/g tissue	**0.25 (0.14–0.28)** 0.22±0.03	**0.08*** (0.06–0.09)** 0.08±0.01	**0 ***&&& (0–0.005)** 0.007±0.01	**0.01 *** (0–0.05)** 0.03±0.02	**0.05 ** (0.01–0.08)** 0.06±0.03

BDL – below detection limits; XO – xanthine oxidase.
** – P < .05 compared to IC values; ** – P < .02 compared to IC values; *** – P < .01 compared to IC values;*
& – P < .05 compared to M+ALL values; && – P < .02 compared to M+ALL values; &&& – P < .01 compared to M+ALL values;
 # – P < .05 compared to PO+ALL values.

Mouse urate transporter URAT1 was identified in the cilia and apical surface of ventricular ependymal cells, GLUT9 was observed in ependymal cells, neurons, and brain capillaries, and these transporters provide uric acid transport into the cell. ABCG2 was identified in the choroid plexus epithelium and brain capillaries, but not in ependymal cells, and its dysfunction in the brain capillary endothelial cells is supposed to inhibit UA excretion into blood (and these transporters are discussed as a promising target for the local increase of uric acid level in brain that is expedient in neurodegenerative disorders) [27]. The latter is of special interest in the context of our results. There data available do not elucidate the changes of this transporter in hypouricemia. At the same time earlier data obtained from the studies of the basolateral membrane of the choroid plexus of the brain ventricles show that adenosine, guanosine, inosine are able to inhibit the transport systems which mainly provide the excretion of purines from the cerebrospinal fluid into the blood [28]. Under the conditions of XO inhibition, the changes in blood and cerebrospinal fluid content of many purines are evident (uric acid precursors level may be logically increased). In particular, adenosine and guanosine concentrations were elevated in the cerebrospinal fluid of mice treated with allopurinol [29]. Thus, the decreased transport of purines out of the brain together with the preserved activity of XO (which activity in brain has been confirmed [26], while the low dose of intragastrically administered ALL in our study possibly resulted in its low availability to the brain tissue) could led to the elevated level of UA within the brain despite the decreased systemic level of this metabolite. This is indirectly supported by elimination of the correlation between uricemia and brain uric acid level: it was seen in intact mice ($\rho = -0.74$, $P > .05$), while in all other groups coefficients were was within the range of –0.11 – +0.10. In PO+ALL group brain uric acid level returned to the value of the intact animals (Table 2), that was not seen in mice receiving GW preparations.

Table 2. The effect of allopurinol and *Aegopodium podagraria* L. preparations on the brain biochemical values of potassium oxonate-treated mice, Q_{50} (Q_{25}–Q_{75}); M±m, n=5–7

		IC	M + ALL	PO + ALL	PO + ALL + EXTR	PO + ALL + TINCT
Content, μM/g tissue	Glutamic acid	**10.7 (10.0–11.8)** 10.9±0.43	**21.4** *** **(21.1–22.2)** 21.4±0.55	**12.0** &&& **(11.9–12.1)** 12.0±0.11	**20.1** ***### **(19.0–20.8)** 20.3±0.68	**22.3** ***### **(21.1–22.9)** 22.9±1.35
	GABA	**1.77 (1.59–1.92)** 1.77±0.09	**0.71** *** **(0.64–0.76)** 0.71±0.05	**1.69** &&& **(1.69–1.77)** 1.69±0.06	**0.77** ***### **(0.72–0.84)** 0.78±0.04	**0.71** ***### **(0.48–0.87)** 0.69±0.10
	Aspartic acid	**3.82 (3.38–4.06)** 3.69±0.18	**5.91** *** **(5.74–6.05)** 5.90±0.10	**3.65** &&& **(3.12–3.79)** 3.64±0.31	**5.27** ***&### **(4.93–5.55)** 5.29±0.20	**6.11** ***### & **(5.76–6.31)** 5.96±0.20
	Uric acid	**0.178 (0.173–0.180)** 0.176±0.003	**0.295** *** **(0.290–0.301)** 0.299±0.007	**0.191** ** &&& **(0.187–0.205)** 0.197±0.007	**0.312** ***&### **(0.307–0.339)** 0.322±0.009	**0.303** ***### **(0.287–0.314)** 0.303±0.010
Serotonin level, nM/ tissue		**118 (115–121)** 118±2,45	**115 (112–117)** 115±1.55	**135** ***&&& **(133–137)** 135±1.44	**130** ***&&&## **(129–131)** 130±0.81	**109** ** &&### **(106–110)** 108±1.32
Spearman's correlation coefficients of ρ between the individual biochemical parameters						
Serotonin – uricemia		**–0.95** *P* = .05	**–0.99** *P* < .001	**+0.10** NS	**–0.03** NS	**+0.15** NS
Serotonin – brain uric acid		**+0.10** NS	**+0.09** NS	**1.0**	**+0.41** NS	**+0.71** *P* = .1
Glutamic acid – aspartic acid		**+0.68** *P* = .09	**+0.09** NS	**–0.20** NS	**–0.89** *P* < .02	**+0.82** *P* < .05
Glutamic acid – brain uric acid		**+0.50** NS	**–0.71** NS	**+0.60** NS	**–0.99** *P* < .001	**–0.14** NS
Aspartic acid – brain uric acid		**+0.70** NS	**+0.60** NS	**+0.60** NS	**+0.92** *P* < .01	**–0.09** NS

NS – not significant.
*** – p<0.02 compared to IC values; *** – p<0.01 compared to IC values;*
& – p<0.05 compared to M +ALL values; && – p<0.02 compared to M +ALL values; &&& – p<0.01 compared to M +ALL values;
 # – p<0.05 compared to PO+ALL values; ## – p<0.02 compared to PO+ALL values; ### – p<0.01 compared to PO+ALL values

The content of the neuroactive aminoacids such as GABA, aspartic and glutamic acids was not changed in the PO+ALL group (glutamic acid level was slightly increased, *P* = .06 compared with the intact control value), while in the groups receiving GW preparations as well as in M+ALL group the decreased level of GABA together with the elevated content of aspartic and glutamic acids were seen (Table 2). Just in these three groups the elevated uric acid level in brain was registered. This attracts attention in the context of the possible stimulatory effect of uric acid on the behavioral responses, cognitive functions, and the motivation [5].

It is has been established that purines modulate the effects of neurotransmitters including GABA and serotonin [30] and serotonin level increased only in the animals which were treated with PO. GW tincture significantly decreased serotonin level, the other studied brain biochemical values did not show changes that could be attributed to GW components influence.

Correlation between GABA and glutamate brain levels was not observed in all groups except for M+ALL group in which it equalled 0.78 (P = .07). Glutamic acid – aspartic acid interrelation was eliminated in M+ALL and PO+ALL groups, the tincture approximated it to the IC group value, while under the influence of the extract the direction of relationship changed. The extract also significantly increased correlations between brain uric acid and glutamic as well as aspartic acid, while the tincture completely eliminated this interrelation.

3.2 The results of the combined open field test

The intragastric administrations together with ALL at lower dose decreased both components of the locomotion activity in the combined open field test (Table 3). In hyperuricemic mice receiving PO per se [13] or in hypouricemic mice receiving ALL as monotherapy at higher doses such changes were not evident (at doses of 10 mg/kg and 39 mg/kg [31]), thus it might reflect the dose-dependence of ALL effects or, probably, the reaction of the animals to the stressory intragastric administration. When ALL was given against the background of PO per se or together with GW extract this decrease was less significant (P =.1 and P = .07 respectively vs the value of M+ALL group). The locomotion activity in animals receiving the tincture did not differ significantly from the value of M+ALL group (Table 2).

Exploratory activity was not changed in any of the groups (Table 3), that is important in the context of the safety of GW preparations use against the background of purine metabolism shifts. Besides, exploratory activity was decreased in hyperuricemic mice receiving PO per se [13], thus, ALL counteracted to this decrease. Uric acid content in brain did not correlate with the total activity in the combined open field. Interestingly, the decrease of locomotor activity (Table 3) were seen in the groups with the reduced GABA content in brain (Table 2).

There were no shifts in the sum of the vegetative manifestations (all of the studied values, such as number of grooming acts, urinations, and fecal boli remained unchanged). Total activity sum was reduced in the groups, in which locomotion activity decreased.

In normouricemic mice GW extract at the dose studied decreased only the number of rearings while the tincture did not influence on this test results [12].

Table 3. The effect of allopurinol and *Aegopodium podagraria* L. preparations on the behavioral responses of potassium oxonate-treated mice in the combined open field test, **Q_{50} (Q_{25}–Q_{75});** M±m; n=6–8

		IC	M + ALL	PO + ALL	PO + ALL + EXTR	PO + ALL + TINCT
Locomotion activity:	Number of squares crossed	**48 (46–54)** 51±3.6	**28 (23–33)***** 29±3.0	**45 (33–48)** 41±5.7	**36 (31–42)*** 40±5.8	**31 (27–38)*** 34±4.6
	Number of rearings	**11 (8–14)** 10±1.7	**5 (2–6)*** 4±1.0	**7 (6–9)** 7±1.6	**4 (4–7)** 6±1.4	**3 (2–4)**** 3±0.5
Number of exploratory nose-pokes		**35 (27–42)** 35±3.1	**32 (31–32)** 31±2.6	**35 (31–36)** 33±1.5	**31 (25–35)** 29±3.0	**30 (26–34)** 32±3.7
Sum of the vegetative manifestations		**0 (0–0.5)** 0.5±0.3	**1.0 (0–1.0)** 0.6±0.2	**0 (0–0)** 0.2±0.2	**0.5 (0–1.8)** 0.8±0.4	**1.0 (0.3–1.0)** 0.8±0.3
Total activity sum		**95 (87–101)** 96±5.7	**63 (59–71)***** 66±6.2	**88 (75–90)** 81±8.0	**75 (71–77)*** **7**6±5.1	**67 (64–78)***** 70±4.5

** – P < .05 compared to IC values; ** – P < .02 compared to IC values; *** – P < .01 compared to IC values.*

3.3 The results of the elevated plus maze test

The studies in the elevated plus maze showed that in all of the groups receiving ALL, except for those additionally treated with the tincture, the mice spent more time in the open arms (Table 4, Fig. 1).

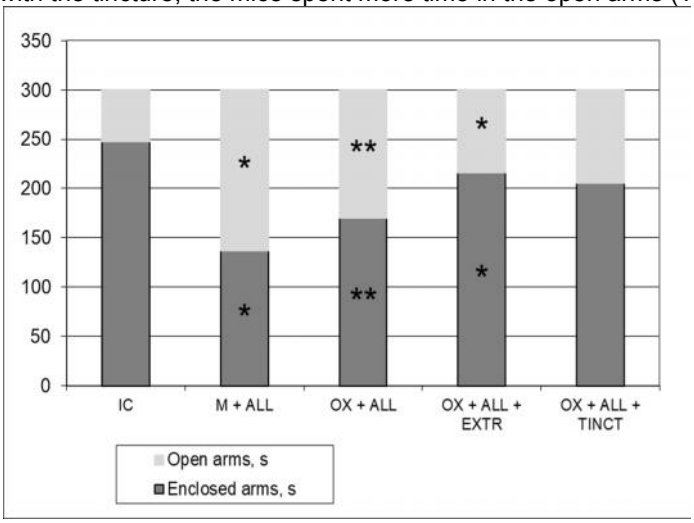

Fig. 1. The effect of allopurinol and *Aegopodium podagraria* L. preparations on the time spent in open and closed arms of the elevated plus maze, seconds

** – P < .05 compared to IC values; ** – P < .02 compared to IC values*

The locomotor activity by the criterion of the total number of arm entries did not differ significantly among the groups. The latence of entry into the enclosed arm was increased in all groups, especially PO+ALL, a higher number of these animals immediately visited the open arm, they visited less enclosed arms and faster entered the open arms (this value tended to statistically significant differences with M+ALL group, *P* =.09 and *P* = .1 in groups PO+ALL and PO+ALL+EXTR respectively). The latter effect was preserved against the background of the extract (as well as the increase in number of mice that immediately visited the open arm and the time spent in the open arms only showed an increment, *P* =.09 vs IC value), while the tincture eliminated it (Table 4).

Table 4. The effect of allopurinol and *Aegopodium podagraria* L. preparations on the behavioral responses of potassium oxonate-treated mice in the elevated plus maze, Q_{50} (Q_{25}–Q_{75}); M±m; n=5–8

	IC	M + ALL	PO + ALL	PO + ALL + EXTR	PO + ALL + TINCT
Latency of entry into the enclosed arm, seconds	**2.5 (1.0–18)** 14±7.6	**18 (8–135)** 76±34	**54 (36–84)*** 61±14	**29 (18–48)** 48±24	**25 (16–39)** 41±20
The number of entries into the enclosed arms	**8 (6–9)** 7±1.1	**4 (3–5)*** 4±0.7	**3 (2–3)*** 3±0.9	**5 (3–7)** 5±1.1	**6 (3–6)** 5±1.0
The number of entries into the open arms	**12 (8–15)** 11±1.8	**9 (6–9)** 8±1.0	**8 (8–9)** 8±1.3	**9 (7–12)** 9±2.0	**7 (4–11)** 8±2.7
The number of maze center crossings	**4 (3–5)** 4±1.0	**4 (3–6)** 5±0.8	**6 (5–7)** 5±0.9	**5 (3–7)** 5±1.0	**6 (2–10)** 6±2.2
Total number of arm entries	**24 (17–30)** 23±3.7	**18 (13–19)** 17±1.7	**16 (15–19)** 17±2.8	**18 (14–24)** 19±3.9	**23 (9–23)** 19±5.5
Latency of entry into the open arm, seconds	**20 (7–35)** 24±7.1	**27 (10–41)** 34±12.7	**6 (4–12)** 11±5.7	**8 (7–19)** 19±9.7	**44 (21–54)**# 86±54

`Time spent in the open arms only, seconds	**21 (12–46)** 32±10	**148 (50–170)**** 119±28	**99 (92–101)**** 98±20	**65 (35–84)** 79±27	**50 (29–71)** 66±31
Time spent in open arms and maze center excepting the latency of entry into the enclosed arm, seconds	**42 (25–59)** 45±11	**60 (42–71)** 79±29	**46 (11–113)** 73±34	**58 (50–68)** 67±15	**57 (45–90)** 62±20
Sum of the vegetative manifestations	**0 (0–1.0)** 0.4±0.2	**0**	**0 (0–0)** 0.2±0.2	**0**	**0 (0–0)** 0.2±0.2
Number of mice that immediately visited the open arm, %	**37,5**	**57,1**	**100***	**83,3**	**40,0**

321 ** – P < .05 compared to IC values; ** – P < .02 compared to IC values*

322 Thus, the effects of the extract are similar to those in the intact normouricemic mice, while the ability
323 of the tincture to reduce anxiety signs registered in normouricemic mice [12] was not evident against
324 the background of ALL and PO. In the group receiving the extract, a positive correlation between the
325 latency of entry into the open arm and uricemia appeared (ρ = +0.97; *P* < .01), that was not present
326 in all of the other groups (and the previously seen correlation between the latency of entry into the
327 enclosed arm and uricemia [13] did not reach the level of statistical significance in this study).
328 Besides, in mice treated with the extract a negative correlation between the latency of entry into the
329 open arm and serotonin content in brain was registered (ρ = –0.90; *P* < .02), in all of the other groups
330 it did not reach a significant level (it was within the range of –0.50 – +0.49). Since this correlation
331 differed between PO+ALL and PO+ALL+EXTR groups, while there were no differences in both
332 serotonin level and the latency of entry into the open arm, the inflence of the extract on serotonin
333 transport, pool or the interrelated neurochemical mechanisms may be supposed (besides, the use of
334 the whole brain homogenate for analysis could not allow registering the changes of the
335 neuromediators in certain regions). One of the main components of the extract are hydroxycinnamic
336 acids and it is notable that ferulic acid is able to exert psychotropic effects through serotonergic
337 system [32]. Furthermore, this effect is registered with the respectively high doses 40 and 80 mg/kg,
338 and the animals may receive the close doses with the extract at a dose of 1 g/kg, but not with the
339 tincture at a dose of 1 ml/kg [9]. The latter decreased serotonin level (Table 2), while the latency of
340 entry into the open arm was maximal just in this group (Table 4).

341 The changes in the preferences of the illuminated and dark compartments may be also attributed to
342 ALL administration since this drug at the same dose reduced the number of mice that immediately
343 visited the enclosed arm and significantly increased the time spent in the open arms [13]. Besides, it
344 has been shown that stress can not only increase anxiety level, but also reduce it depending on the
345 timing of the stressor [33], and the situation of the chronic stress was highly possible in animals
346 undergoing the constant intragastric administrations. As to the extract partial efficacy, it is unlikely that
347 the slight increase in uricemia could influence on the anxiety level, so this effect could be attributed to
348 its components direct effects. In this context the data concerning the anxiolytic effect of chlorogenic
349 acid are of special interest (and this effect was seen in mice after the administration of the substance
350 at a dose of 20 mg/kg [34], while the quantity of hydroxicinnamic acids that the animal receives with
351 the dose of 1 /kg reaches 53 mg/kg [9]).

352
353 **3.4 The results of depressivity signs evaluation**
354
355 In mice receiving ALL at low dose (and undergoing chronic intragastric administrations) a signifcant
356 increase in depressivity level was seen by criterion of the duration of immobility in the tail suspension
357 test (Fig. 2). These changes were reduced in animals receiving PO, and in mice receiving GW
358 preparations this value had no statistical differences from IC value (the effect was especially stable in

mice receiving the tincture). The number of fecal boli during the test did not vary between the groups (data not shown).

It has been shown previously that ALL per se at a dose of 10 mg/kg does not change depressivity level [13], in contrast to data confirming the reduction in the immobility period in mice chronically treated with ALL at a dose of 39 mg/kg (the similar effect was exerted by febuxostat) [31]. On the other hand, the decreased locomotion activity and possibly stress-induced changes in anxiety level might be logically combined with the increase in depressivity level. Besides, the above-mentioned changes of uric acid precursors are possible, and in the context of ALL influence on the CNS special attention is given to the changes in adenosine level [35]. It is known that adenosine and inhibitors of its transport increase immobility of animals in the tests of behavioural despair [36]. The involvement of the serotoninergic system into the depressivity changes is also possible but it was not influenced by GW preparations (proceeding from the correlation coefficients between brain serotonin level and immobility time: the negative interrelationship inherent in intact mice, ρ = –1.0 was eliminated in all other groups, in PO+ALL group it equalled –0.50; *P* > .05, in the other groups it was within the range of –0.21 – +0.20).

GW extract and tincture did not exert an antidepressive effect in normouricemic male mice (while in female animals it was present after administration of the extract at a tenfold lower dose) [12]. It is important that this effect was evident in our study – in animals with the increased depressivity signs. In accordance with the previous results [13], a positive correlation between the duration of immobility and uricemia (ρ = +0.71, *P* = .07) was present in IC group. It was eliminated in M+ALL group (ρ = –0.20, *P* > .05), while in the group receiving ALL and PO interconnection direction changed (ρ = –0.80, *P* = .1), that was further enhanced by the extract (ρ = –0.99, p<0.001) but not by the tincture (ρ = –0.50, P > .05).

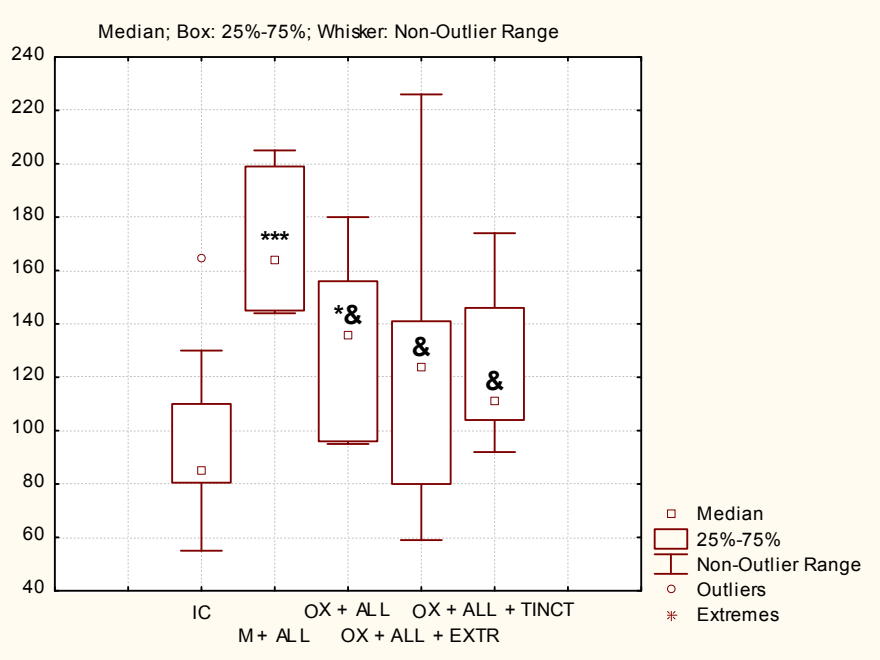

Fig. 2. The effect of allopurinol and *Aegopodium podagraria* L. preparations on the duration of immobility of potassium oxonate-treated mice in the tail suspension test, seconds

** – P < .05 compared to IC values; *** – P < .01 compared to IC values; & – P < .05 compared to M +ALL values*

Together with the changes in purine metabolism, direct influence of GW components on the CNS is possible, and hydroxycinnamic acids are of special interest. Much evidence exists about the beneficial

activity of chlorogenic acid (which takes part in the development of the favourable central effects of coffee) including antidepressive as well as anxiolytic properties in different experimental models. Dose dependence is inherent in some of the effects of the hydroxycinnamic acids [37,38.]. Further research is expected to clarify whether the modulatory effect on the purinergic mechanisms is inherent in these compounds.

3.5 The results of the physical endurance evaluation

The physical endurance by criterion of duration of swimming in the weight-loading forced swimming test was reduced in all of the groups receiving ALL (Fig. 3). This decrease was especially significant in M+ALL group, while the tincture approximated this value to the level of the IC.

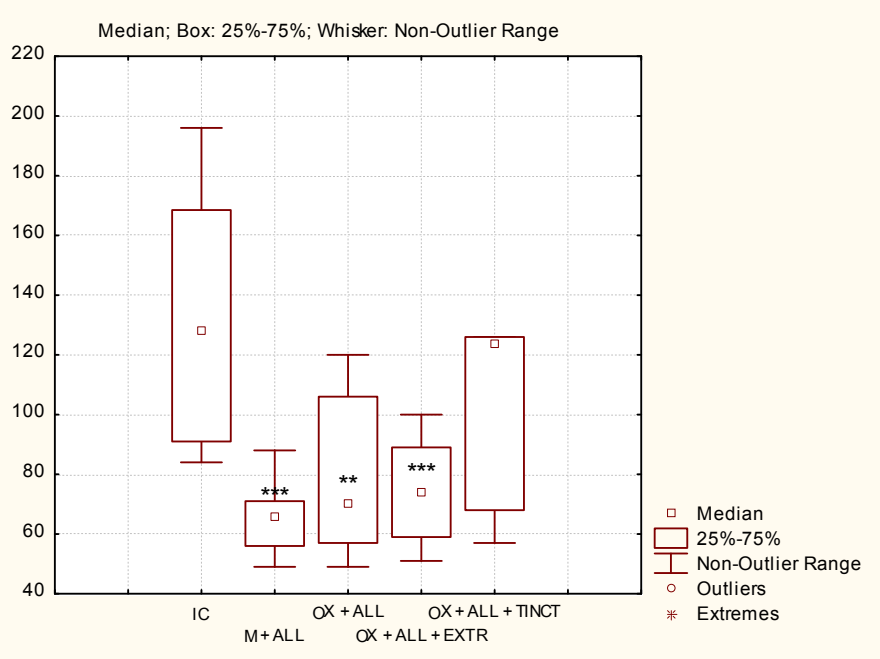

Fig. 3. The effect of allopurinol and *Aegopodium podagraria* L. preparations on the physical endurance of potassium oxonate-treated mice (duration of swimming in the weight-loading forced swimming test), seconds

*** – P < .02 compared to IC values; *** – P < .01 compared to IC values.*

Body weight of all mice was not changed (only in animals receiving the extract there was a slight but significant decrease compared to the IC value), thus the loading did not differ significantly among the groups. In our previous experiments, ALL per se at a dose of 10 mg/kg did not reduced the duration of swimming, on the contrary, there was a tendency to the increase in this value [13], while the other authors have demonstrated the reducing of the swimming duration in rats treated with xanthine oxidase inhibitor [39]. The open space swimming test results may be influenced by the state of peripheral metabolism and ALL is known to support anabolic processes through the increased the availability of hypoxanthine for the synthesis of nucleotides [40]. Nevertheless, this effect was not evident and the decrease in swimming duration might be caused by the chronic stress which is known to reduce physical activity in most cases [41].

The role of uricemia reduction is also possible since the correlations between the duration of swimming and uricemia changed in a similar direction as did correlation between the duration of immobility and uricemia discussed above (a positive correlation in IC group that was eliminated in M+ALL group, while in the group receiving ALL and PO interconnection direction changed to negative), and at both groups receiving GW preparations this correlation was not significant. The tincture also normalized glutamic acid – aspartic acid correlation (described above) that also may be associated with depressivity changes. Thus, the role of the direct action of GW components might be expected and the dose-dependent effect is possible at that (regarding the significantly higher content

of hydroxicinnamic acids in the extract). The tincture in previous experiments did not change the physical endurance, while the extract increased it at a significantly lower dose [42]. It seems to be favourable that the effect is present just in the model with the decreased physical performance.

3.6 The results of the extrapolation escape task

There were no differences in all the measures taken in the extrapolation escape task, namely the latency of escape and number of animals in each group that solved the task (data not shown). Thus, the cognitive functions remained unaltered demonstrating the well known brain plasticity. These aspects are favourably combined with the absence of the decrease in the exploratory behaviour in the combined open field test (discussed above). Besides, it is important that in hypouricemic mice GW drugs do not worsen the results of this test (an ambiguous effect was seen previously in rats [42]).

4. CONCLUSIONS

1. Goutweed extract (1 g/kg intragastrically) and goutweed tincture (1 ml/kg intragastrically) do not counteract the inhibitory influence of allopurinol on xanthine oxidase in mice receving potassium oxonate, uric acid concentration in the liver is lower in mice receiving goutweed preparations (especially the tincture) which is accompanied with slightly elevated uricemia allowing to suggest the influence of goutweed components on the transport of uric acid, while uric acid level in the kidney is not changed by goutweed preparations.

2. Brain uric acid level remains unchanged in mice receving potassium oxonate and allopurinol, while goutweed extract and tincture are given against such background, it is significantly increased. The same elevation was seen in mice receiving ALL at a low dose allowing to suppose the decreased transport of uric acid out of the brain. In the groups where brain uric acid is elevated, the decrease in GABA content and the increment in aspartic and glutamic acids are seen. Serotonin level in brain is elevated in mice receving potassium oxonate and allopurinol, the extract decreases it slightly, and the tincture – significantly, while allopurinol per se at a low dose does not change this mediator level.

3. Allopurinol administration to mice receving potassium oxonate leads to the less significant decrease in the locomotion activity compared with the treatment with allopurinol per se at low dose. When goutweed extract is administered to mice receiving allopurinol and potasssium oxonate, this phenomenon is partially maintained, while the tincture under such conditions eliminates it and the locomotion activity does not differ significantly from the value of the group treated with allopurinol per se at low dose.

4. Goutweed tincture eliminates the increase in the duration of stay in the open arms of the elevated plus maze, which is caused by allopurinol against the background of potassium oxonate (the extract causes changes in the same direction, but they are less pronounced and do not reach the level of statistical significance). The tincture also approximates the number of mice that immediately visit the open arm to the value of the intact control.

5. Goutweed extract and the tincture decrease depressivity level by criterion of the duration of immobility in the tail suspension test (this value has no statistical differences from the value of the intact mice). The tincture also restores physical endurance by criterion of duration of swimming in the weight-loading forced swimming test, which is decreased in all other groups receiving allopurinol

ETHICAL APPROVAL

All 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.

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