The influence of *Aegopodium podagraria L.* extract and tincture on behavioural reactions of random-bred mice

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ABSTRACT

Aegopodium podagraria is used as a sedative in traditional medicine. The effect of its aerial part extract and tincture on levels of depression and anxiety, as well as on locomotor activity, exploratory behaviour and memory of male and female mice was investigated. The extract showed dose-dependent and sex specific antidepressive effect (at a dose of 100 mg/kg but not at a dose of 1 g/kg in female mice) with the worsening of the results of the passive avoidance test. The extract at a dose of 100 mg/kg tended to reduce anxiety signs in the animals of both sexes, in male mice such reduction was also seen under the influence of the extract at a dose of 1 g/kg and the tincture at doses of 1 and 5 ml/kg (the latter did not considerably changed the other parameters measured). The results indicate favourable central activity of A. podagraria extract in mice.

Keywords: *Aegopodium podagraria L.*, central nervous system, anxiolytic effect, antidepressant effect, mice.
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INTRODUCTION

Anxiety disorders present a serious problem for modern populations because of high prevalence, often chronic-recurrent course and quality of life worsening [1,2]. According to pharmacoepidemiological data there is a high prescription rate for anxiolytic drugs all over the world [3]. Among the antianxiety agents benzodiazepines have the most substantial evidence of efficacy, still they may cause side effects such as physical dependence, withdrawal symptoms and sedation [1,4–6]. Antidepressant drugs are also used in anxiety disorders being the treatment of choice for long-term management of chronic anxiety, especially in the presence of depressive symptoms. They are also not devoid of side effects, such as somnolence, nausea, sexual dysfunction, insomnia, gastrointestinal problems (for selective serotonin reuptake inhibitors) and sedation, orthostatic hypotension, anticholinergic effects, weight gain, high toxicity at overdosage (for tricyclic antidepressants) [1,5,6]. In spite of introduction of novel schemes of treatment including combinations of antidepressants and benzodiazepines, remission rates are still only about 40% so other approaches should be developed [1]. Therefore, the search for effective and safe drugs with anxiolytic and antidepressive properties is being conducted nowadays. The interest in herbal drugs has increased lately because the most of them are characterized by high safety along with polymodal pharmacological action due to the complex composition [7–9].

Aegopodium podagraria L. (goutweed) is an ubiquitous perennial herb of the *Apiaceae* family. It is indigenous to Europe, Siberia, the Caucasus, Kazakhstan and Central Asia mountainous regions, naturalized in North America and Australia. According to ethnobotanical information, it is consumed as vegetable (leaves of young plants) and widely used for the treatment of gout and related states, rheumatism, kidney and bladder diseases, gastrointestinal tract disorders [10–12]. The Latin species name was given to the plant by Linnaeus in accordance with its use in gout; the expediency of this approach has been confirmed by pharmacological research. Hypouricemic and uricosuric action was established in preparations obtained from *A. podagraria* aerial part [13–16]. In addition to the influence on uric acid metabolism that is principal in gout and hyperuricemia, *A. podagraria* drugs suppress inflammation [15,17], this effect is partially attributed to polyacetylene falcarindiol possessing marked COX-1 inhibitory activity [18]. Besides, pharmacological research has confirmed nephroprotective, hepatoprotective, hypoglycaemic properties as

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well as low toxicity level of the drugs obtained from *A. podagraria* aerial part [14,15,19,20]. Sedative effect is among the properties of *A. podagraria* mentioned in traditional medicine [10,12], but there is no data on this effect scientific verification. At the same time, hydroxycinnamic acids (caffeic, chlorogenic), flavonoids, coumarins, polyacetylene compounds (falcarindiol), essential oil components, micro- and macroelements were identified in the *A. podagraria* raw material [13,14,17,21–24]. Many of these substances possess proven influence on the functional state of the CNS. For instance, caffeic acid exerts antidepressive and anxiolytic effects [25], falcarindiol and ferulic acid exhibit affinity towards 5-HT7 receptors [26]; sufficient intake of potassium and magnesium decreases the level of depression in animals [27] and is correlated with overall psychiatric functioning in adults with mood disorders [28].

Moreover, the widespread use of *A. podagraria* in traditional medicine makes it necessary to investigate its possible impact on health. In particular, psychotropic effects may accompany the prolonged usage of this plant for the treatment of chronic diseases. It is also important to study both aqueous and alcoholic extracts (namely, *A. podagraria* dry extract and the tincture) because they have significant differences in pharmacological effects. The tincture has more pronounced influence on glucose metabolism and inflammation process, and it is characterized by the presence of polar and nonpolar compounds. Still the latter cannot be considered among the active components of goutweed as they may cause unfavourable metabolic effects; and *A. podagraria* dry extract surpasses the tincture by protective activity under the conditions of kidney and liver injury. These drugs also differ in the mechanisms of their influence on uric acid metabolism [13,15,20]. At the same time the links between uric acid exchange and the CNS functional state cannot be excluded (although it is not common approach in pharmacological studies). Thus, apart from widely discussed role of hyperuricemia in the development of metabolic syndrome and hypertension, this state is associated with neuroprotection and possible cognition enhancement [29,30]. It has recently been shown that hyperuricemic mice demonstrate reduced signs of anxiety and level of depression [31], juvenile hyperuricemic rats show increased locomotor activity [32]. Interestingly, the widespread hypouricemic drug allopurinol as well as febuxostat expressed significant antidepressant like effect [33] and allopurinol reduced anxiety that could be associated with uric acid precursors concentrations changes [31]. Still the relationship between antihyperuricemic and psychotropic activity of the herbal drugs (including goutweed preparations) is not elucidated in the data in the literature. As the influence of the course administration of goutweed aerial part on uricemia has not been studied in female mice; and there is no data concerning their influence on the CNS functional state, we aimed to investigate these effects just in female mice.

A proper evaluation of the psychotropic effects of the investigated herbal drug presupposes the comparison with the reference drug with a well-documented pharmacological activity. Such drugs are certainly found among herbal preparations. Thus, extracts of *Passiflora incarnata L.* (passionflower) demonstrate anxiolytic and sedative activity, extracts of *Hypericum perforatum L.* (St. John's wort) show antidepressive activity; these properties are not only seen in an animal behavioural paradigms [34,35], but are of clinical significance and the drugs are present at the pharmaceutical markets [7,9]. The chemical constituents which mediate psychotropic activity are identified in these plants [7]. Besides, it is reasonable to investigate herbal drugs using the route of administration that is of clinical significance, namely peroral route that is also typical for *P. incarnata L.* and *H. perforatum L.* extracts [34,35]. So *P. incarnata L.* and *H. perforatum L.* extracts were chosen to compare with *A. podagraria* preparations. Reference drugs were adminisrered to male mice for the consistent comparison of the results with the present in literature data, mainly obtained from male laboratory animals.

At the same time, sex-specific differences in psychotropic drugs effects have attracted much attention recently [36–38]. Still the efficacy of herbal drugs in this context is poorly understood. Considering their polytropic metabolic effects such investigations seem to be quite perspective.

Therefore, the aim of this study was to determine the impact of *A. podagraria* extract and tincture on levels of depression and anxiety, as well as on locomotor activity and exploratory behaviour of intact randombred mice with the ascertainment of the possible differences between male and female animals. *A. podagraria* effects were compared with the effects of *Hypericum perforatum L.* (St. John's wort) and *Passiflora incarnata L.* Additionally we addressed the influence of *A. podagraria* preparations on memory and studied the possible relationship of influence on the central nervous system with changes in uricemia.

EXPERIMENTAL SECTION

Plant material
The aerial parts of *A. podagraria* were collected from the natural population in Kharkiv region (Ukraine) in June. They were identified by Ass. Prof. Dr. S.I. Stepanova (National University of Pharmacy, Kharkiv, Ukraine). 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.

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 accompanying occasional shaking and stirring. 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 *A. podagraria* dry extract and tincture obtaining was in accordance with the requirements of State Pharmacopoeia of Ukraine and was described previously by our group [16,19,39].

Drugs and chemicals
Hypericum perforatum L. and *Passiflora incarnata L.* extracts were used as the reference drugs. *P. incarnata* extract ("Alora" syrup from NOBEL ILAC Sanayii ve Ticaret A.S., Turkey) was administered at a dose of 300 mg/kg that possesses anxiolytic properties according to [35]. *H. perforatum* extract ("Deprivit" from Kyiv Vitamin Factory, Ukraine) was administered at a dose of 100 mg/kg that showed significant antidepressant activity in experiments [34]. *A. podagraria* extract and *P. incarnata* syrup were administered in the form of aqueous solution. *H. perforatum* extract was administered in the form of suspension stabilized by polysorbate 80. Alcohol was removed from *A. podagraria* tincture before administration. All these preparations were made *ex tempore*.

Animal groups and treatment
Adult random-bred female and male mice (body weight 20–24 and 16–18 grams respectively) 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 1 week of acclimation, **female mice** were randomly assigned to 5 groups:
Group I: intact control (n=8);
Group II: *A. podagraria* extract, 100 mg/kg (n=7);
Group III: *A. podagraria* extract, 1 g/kg (n=8);
Group IV: *A. podagraria* tincture, 1 ml/kg (n=6);
Group V: *A. podagraria* tincture, 5 ml/kg (n=7).

After 1 week of acclimation, **male mice** were randomly assigned to 7 groups:
Group I: intact control (n=8);
Group II: *A. podagraria* extract, 100 mg/kg (n=9);
Group III: *A. podagraria* extract, 1 g/kg (n=8);
Group IV: *A. podagraria* tincture, 1 ml/kg (n=8);
Group V: *A. podagraria* tincture, 5 ml/kg (n=8):
Group VI: *P. incarnata* extract, 300 mg/kg (n=7);
Group VI: *H. perforatum* extract, 100 mg/kg (n=7);

Herbal drugs were administered intragastrically once a day. The mice of the intact control groups received intragastrically distilled water by the similar scheme. The amount of fluid that the mice in all groups received was similar and equalled 0.2 ml/20 g. Beginning from the 5th day psychopharmacological tests were carried out. Locomotor activity and exploratory behaviour were studied in a combined open-field test, the level of anxiety – in the elevated plus maze test and the level of depression – in the tail suspension test. As this test influences depression level (Vogel, 2008), it was conducted as a final one. In addition, we used the passive avoidance test to evaluate memory processes in animals treated with *A. podagraria drugs*. 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.

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Behavioural tests
Combined open field
The mice were observed in an open field arena, $22 \times 22 \times 11$ ($L \times W \times H$) 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.

Elevated plus maze
The elevated plus maze apparatus (Vogel, 2008) was made of plastic, glass and wood. It consisted of two brightly-lit open arms (glass surface), 10×50 ($L \times W$), two opposed enclosed arms $10 \times 10 \times 50$ ($L \times W \times H$), 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, such factors as latency of entry into the open arm and into the enclosed arm, the number of times the maze center was crossed were registered. These factors are widely used for the detailed characteristic of rodents' anxiety [40–43]. Also the number of fecal boli and urinations was registered.

Tail suspension test
The mice were suspended on the support by adhesive tape placed approximately 1 cm from the tip of the tail [44]. 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.

Passive avoidance test (trial-to-criteria inhibitory avoidance)
A rectangular box with a 20×15 cm grid floor and 15 cm high walls was used. In the centre of one wall there was a 5×7 cm opening (that could be closed with a transparent sliding door) connecting this compartment to the box of the same size with dark walls, electrifiable grid floor and removable ceiling. The mouse was placed in the illuminated box facing the wall opposite to the opening. After an entry to the dark box the sliding door was closed and mild footshock was delivered that was enough for the reflex formation. No factors producing amnesia were applied. Retention of the test was measured 24 hours later. The animals were placed in the lighted area, the door opened and the latency to step with the four paws into the dark area was recorded. Mice that did not entered the dark box within 3 min were removed from the box, and the latency was recorded as 180 s. Latency after 24 h and the % of avoidance (percent of animals that had acquired the training criterion and had not entered the dark box during 180 s) were recorded [44,45]. Additional measures such as the number of unfinished attempts to entry into the dark box (without the placement of all four paws into it) and the latency of the first unfinished attempt were used because they appeared to be informative criteria for anxiolytic herbal drugs characteristic [41].

Uricemia determination
To determine the possible relationship of influence on the central nervous system with changes in purine metabolism uricemia was measured in female mice (uricemia in male mice was not determined as the previous research have shown no changes in this index in male mice [13]). 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 determined by the uricase method [46] with commercially-available kit (Spine-Lab, Ukraine).

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 [47]) 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.

RESULTS

As shown in Table 1 and Table 2, all investigated preparations did not considerably change the results of the combined open field test. Both doses of the tincture and the extract at a dose of 100 mg/kg did not influence locomotor activity and exploratory behaviour of female mice. In female mice, the number of squares crossed was slightly decreased against the background of the extract at a dose of 1 g/kg; the changes were statistically significant compared with the value of animals treated with the low dose of extract but not with the intact control value. In male mice the same tendency failed to reach statistical significance. *A. podagraria* extract in both doses also reduced the

number of times the male animals reared. Total activity sum tended to decrease under the influence of the high dose of extract in female animals and in male mice receiving both doses of the extract. As to emotional and vegetative manifestations, grooming acts and urinations were observed only in certain female mice both in intact control group and in animals receiving *A. podagraria* drugs. The number of fecal boli was slightly augmented in male and female mice treated with the extract at a dose of 1 g/kg and the tincture at a dose of 1 ml/kg. In male animals all *A. podagraria* preparations as well as *H. perforatum* extract increased the number of grooming acts (statistically significant changes in groups of animals treated with *A. podagraria* extract at the lower dose, *A. podagraria* tincture at the high dose and *H. perforatum* extract). So *A. podagraria* extract at both doses augmented the sum of vegetative manifestations in male mice that was more expressed than the changes caused by *A. podagraria* tincture at a dose of 1 ml/kg and *H. perforatum* extract. Only slight increase in locomotor activity and total activity sum was seen under the influence of *P. incarnata* extract.

Table 1. The effect of goutweed drugs on the behavioral responses of female mice in the combined open field test; M±m; Q$_{50}$ (Q$_{25}$–Q$_{75}$)

Measures		Intact control, n=8	Goutweed extract, 100 mg/kg, n=7	Goutweed extract, 1 g/kg, n=8	Goutweed tincture, 1 ml/kg, n=7	Goutweed tincture, 5 ml/kg, n=7
Locomotion activity	Number of squares crossed	**54±7,5** 51 (44–60)	**50±3.5** 55 (45–56)	**40±4.7$^{#}$** 45 (27–49)	**49±3.9** 46 (43–55)	**49±6.9** 43 (37–59)
	Number of times the animal reared	**6±1.5** 5 (4–7)	**8±2.3** 7 (4–12)	**6±1.6** 5 (4–7)	**6±1.0** 6 (4–8)	**4±1.4** 5 (1–7)
Number of exploratory nose-pokes		**20±2.4** 17 (14–27)	**16±1.2** 16 (13–19)	**16±1.7** 17 (13–20)	**21±2.1** 22 (19–24)	**17±1.1** 19 (15–19)
Emotional and vegetative manifestations	Number of grooming acts	0.13	0	0.13	0.14	0.14
	Number of fecal boli	**0.8±0.4** 0.5 (0–1)	**0.9±0.4** 1.0 (0–1)	**1.3±0.3** 1.0 (1–2)	**1.4±0.6** 1.0 (0–2.5)	**0.6±0.3** 0 (0–1)
	Number of urinations	0	0	0	0.14	0
	Sum of vegetative manifestations	**0.9±0.5** 0.5 (0–1)	**0.9±0.4** 1.0 (0–1)	**1.4±0.2** 1.0 (1–2)	**1.7±0.6** 1.0 (0–3)	**0.7±0.3** 1.0 (0–1)
Total activity sum		**80±10.5** 76 (64–89)	**75±5.6** 76 (69–85)	**63±7.4** 68 (47–77)	**77±5.5** 76 (71–84)	**71±7.5** 75 (58–83)

*Results are **means** ± **SEM**; median (lower quartile – upper quartile).*
 # – Significant at p<0.05 compared with the group of animals receiving goutweed extract at a dose of 100 mg/kg

Table 2. The effect of goutweed and reference drugs on the behavioral responses of male mice in the combined open field test; M±m; Q$_{50}$ (Q$_{25}$–Q$_{75}$)

Measures		Intact control, n=8	Goutweed extract, 100 mg/kg, n=9	Goutweed extract, 1 g/kg, n=7	Goutweed tincture, 1 ml/kg, n=8	Goutweed tincture, 5 ml/kg, n=8	Passiflora extract, 300 mg/kg, n=7	Hypericum extract, 100 mg/kg, n=7
Locomotion activity	Number of squares crossed	**35±5,7** 34 (25–41)	**29±4,1** 31 (21–34)	**27±6,1** 23 (19–29)	**36±3,1** 38 (34–40)	**34±7,7** 36 (12–52)	**46±10,0** 45 (34–53)	**32±4,5** 36 (26–37)
	Number of times the animal reared	**2±0,7** 2 (0–3)	**0,7±0,3$^{&}$** 0 (0–2)	**0,7±0,3$^{&}$** 1 (0–1)	**2±0,7** 2 (1–4)	**4±1,6** 2 (0–6)	**2±1,0** 1 (0–4)	**2±0,8** 2 (1–3)
Number of exploratory nose-pokes		**16±1,8** 15 (12–19)	**15±1,5** 14 (11–18)	**16±1,4** 15 (13–18)	**18±2,0** 18 (14–22)	**16±2,6** 17 (14–17)	**19±4,0** 16 (13–24)	**14±3,1** 11 (9–21)
Emotional and vegetative manifestations	Number of grooming acts	0	**0,6±0,2*** 1 (0–1)	**0,3±0,2** 0 (0–1)	**0,3±0,2** 0 (0–0,3)	**0,4±0,2*** 0 (0–1)	0,14	**0,4±0,2*** 0 (0–1)
	Number of fecal boli	**0,3±0,2** 0 (0–0,3)	**0,3±0,2** 0 (0–1)	**0,7±0,4** 0 (0–1)	**0,5±0,2** 0,5 (0–1)	0,13	**0,3±0,2** 0 (0–0,5)	**0,3±0,2** 0 (0–0,5)
	Number of urinations	0	0$^{#}$	**0,4±0,2*** 0 (0–1)	0	0	0$^{#}$	0$^{#}$
	Sum of vegetative manifestations	**0,3±0,2** 0 (0–0,3)	**0,9±0,2*** 1 (1–1)	**1,4±0,5*** 1 (0,5–2)	**0,8±0,2** 1 (0,8–1)	**0,5±0,2** 0,5 (0–1)	**0,4±0,3** 0 (0–0,5)	**0,7±0,3** 1 (0–1)
Total activity sum		**53±7,8** 48 (41–63)	**45±5,2** 48 (41–55)	**45±6,8** 38 (35–49)	**57±5,2** 61 (51–64)	**54±10,3** 55 (28–82)	**67±13,6** 66 (53–77)	**49±6,8** 48 (38–60)

*Results are **means** ± **SEM**; median (lower quartile – upper quartile). 1. * – Significant at p<0.05 compared with intact control group;*
2. # – Significant at p<0.05 compared with the group of animals receiving goutweed extract at a dose of 1 g/kg;
3. & – Significant at p<0.05 compared with the group of animals receiving Hypericum extract.

Table 3. The effect of goutweed and reference drugs on the number of mice that immediately visited the open arm of the elevated plus maze, % (absolute quantity)

MALE MICE					
Intact control, n=8	Goutweed extract, 100 mg/kg, n=9	Goutweed extract, 1 g/kg, n=8	Goutweed tincture, 1 ml/kg, n=8	Goutweed tincture, 5 ml/kg, n=7	Passiflora extract, 300 mg/kg, n=7	Hypericum extract, 100 mg/kg, n=7
13 (1/8)	**78** (7/9)[**]	**63** (5/8)[*]	**63** (5/8)[*]	**38** (3/8)	**71** (5/7)[**]	**71** (5/7)[**]
FEMALE MICE						
Intact control, n=8	Goutweed extract, 100 mg/kg, n=6	Goutweed extract, 1 g/kg, n=8	Goutweed tincture, 1 ml/kg, n=7	Goutweed tincture, 5 ml/kg, n=6	n/s	n/s
13 (1/8)	**67** (4/6)[*]	**38** (3/8)	**57** (4/7)[*]	**67** (4/6)[*]		

n/s – not studied

* – Significant at p<0.05 compared with intact control group;

** – Significant at p<0.02 compared with intact control group;

Table 4. The effect of goutweed drugs on the results of the passive avoidance test in female mice; M±m; Q_{50} (Q_{25}–Q_{75})

№	Group	Latency of entry into the dark box after 24 h, s	% of avoidance (absolute quantity)	Number of unfinished attempts to entry into the dark box	Latency of the first unfinished attempt, s
1	Intact control, n=7	**124±29** 180 (73–180)	**57** (4/7)	**3.5±0.8** 4 (2–5)	**51±25** 21 (13–72)
2	Goutweed extract, 100 mg/kg, n=7	**101±21** 95 (64–139)	**14** (1/7)[*]	**2.6±0.8** 2 (1–4)	**36±13** 29 (27–37)
3	Goutweed extract, 1 g/kg, n=8	**143±22** 180 (131–180)	**63** (5/8)[#]	**3.4±0.9** 3 (2–5)	**23±2.1** 25 (25–25)
4	Goutweed tincture, 1 ml/kg, n=6	**79±32** 42 (21–148)	**33** (2/6)	**3.3±0.9** 3 (3–4)	**68±43** 35 (26–94)
5	Goutweed tincture, 5 ml/kg, n=7	**119±29** 180 (43–180)	**57** (4/7)	**3.4±1.4** 2 (2–3)	**28±9.3** 19 (15–28)

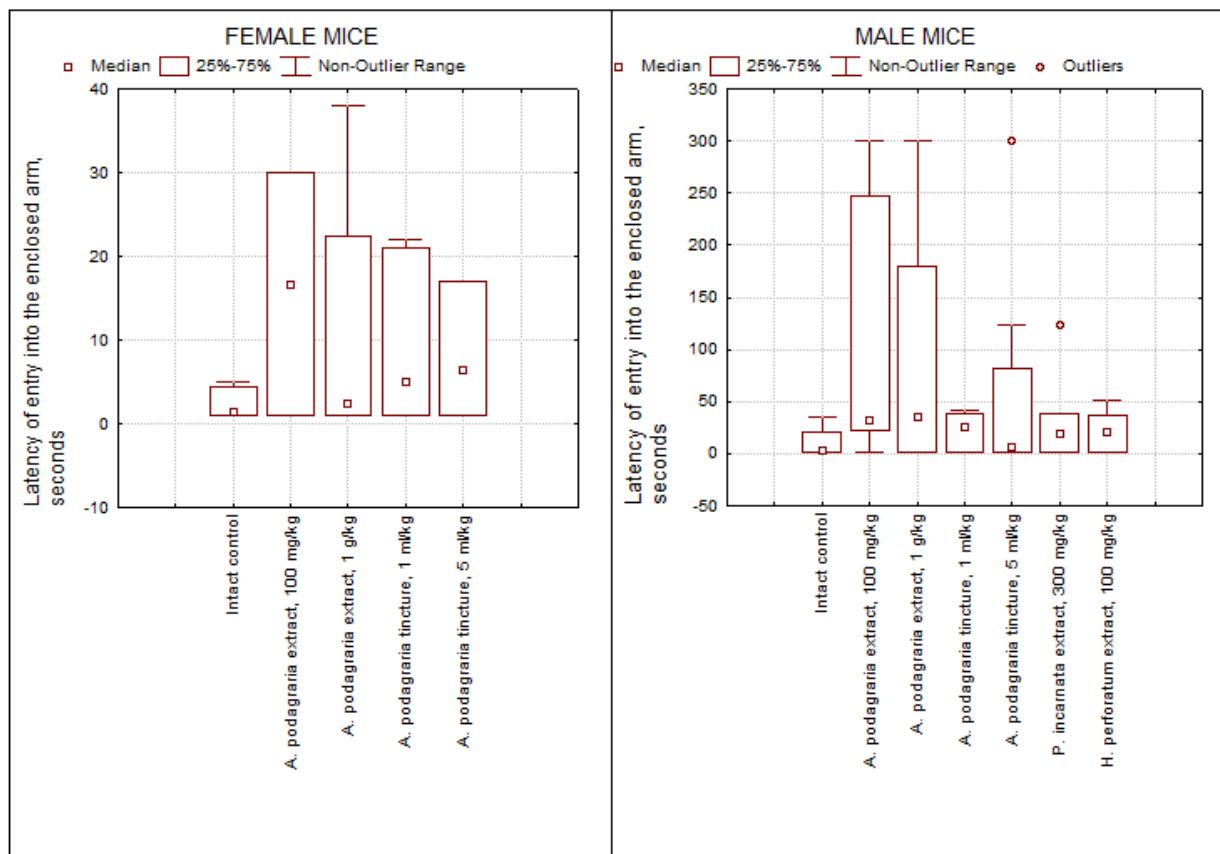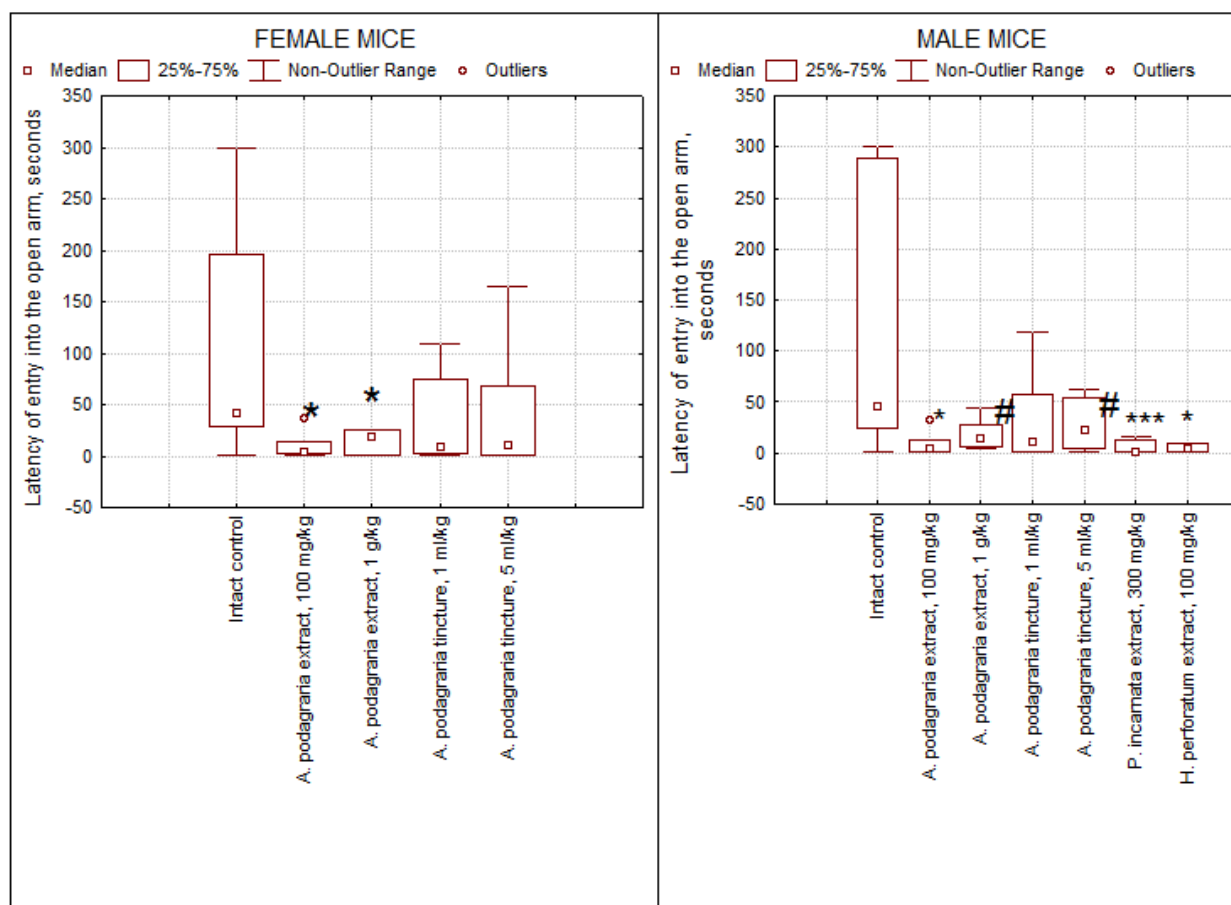*Results are **means** ± **SEM**; median (lower quartile – upper quartile).*

* – Significant at p<0.05 compared with intact control;

 # – Significant at p<0.05 compared with the group of animals receiving goutweed extract at a dose of 100 mg/kg

Figure 1. The effect of goutweed and reference drugs on the latency of entry into the enclosed arm of the elevated plus maze

Figure 2. The effect of goutweed and reference drugs on the latency of entry into the open arm of the elevated plus maze

* – Significant at p<0.05 compared with intact control group;
*** – Significant at p<0.001 compared with intact control group;
 # – Significant at p<0.05 compared with the group of animals receiving Passiflora extract

Table 5. The effect of goutweed drugs on the results of the passive avoidance test in male mice; **M±m;** Q_{50} (Q_{25}–Q_{75})

№	Group	Latency of entry into the dark box after 24 h, s	% of avoidance (absolute quantity)	Number of unfinished attempts to entry into the dark box	Latency of the first unfinished attempt, s
1	Intact control, n=8	**110±24** 108 (62–180)	**38** (3/8)	**2,8±0,7** 2 (2–4)	**50±19** 42 (29–64)
2	Goutweed extract, 100 mg/kg, n=8	**92±27** 66 (27–180)	**25** (2/8)	**1,9±0,3** 2 (1–2)	**30±15** 12 (2–36)
3	Goutweed extract, 1 g/kg, n=8	**114±27** 145 (42–180)	**50** (4/8)	**2,5±1,0** 1,5 (1–3)	**18±4,6** 20 (10–27)
4	Goutweed tincture, 1 ml/kg, n=8	**115±26** 140 (56–180)	**50** (4/8)	**2,5±1,0** 1,5 (1–3)	**34±5,4** 31 (25–38)
5	Goutweed tincture, 5 ml/kg, n=8	**103±26** 112 (36–170)	**25** (2/8)	**2,6±0,9** 2 (1–3)	**53±22** 29 (8–82)

*Results are **means ± SEM**; median (lower quartile – upper quartile).*

In the elevated plus maze latency of entry into the enclosed arm displayed a pronounced tendency towards augmentation in male animals receiving *A. podagraria* extract, the less expressed changes were registered in all the other groups, including the mice treated with the reference drugs (Fig. 1). In female mice a similar tendency was evident under the influence of the extract at a dose of 100 mg/kg. It should be noted that percentiles are especially informative indicators in this case because of the absence of normal distribution and marked inter-individual differences (the last-mentioned led to the absence of statistically significant differences in latency of entry into the enclosed arm). At the same time, the latency of entry into the open arm decreased significantly in animals of both sexes treated with *A. podagraria* extract (Fig 2). The high dose of the extract did not surpass the lower by this action. All the investigated drugs, except for the extract at high dose, tended to increase the total number of arm entries (data not shown, there were no statistically significant differences between the groups) and in female mice the number of entries into the open arms depended on the extract dose: this value (Q_{50} (Q_{25}–Q_{75})) equalled 9 (8–9) and 5 (3–6) in animals receiving the drug at a dose of 1 g/kg and 100 mg/kg respectively (p<0,05), and in control group it equalled 5 (3–6). The same situation (not reaching the level of statistical significance) was seen in male

mice. The faster entry into the open arms was also observed in animals receiving *A. podagraria* tincture and the extracts of *H. perforatum* and, particularly, *P. incarnata* (Fig 2).

Figure 3. The effect of goutweed and reference drugs on the time spent in the open arms and center of the elevated plus maze
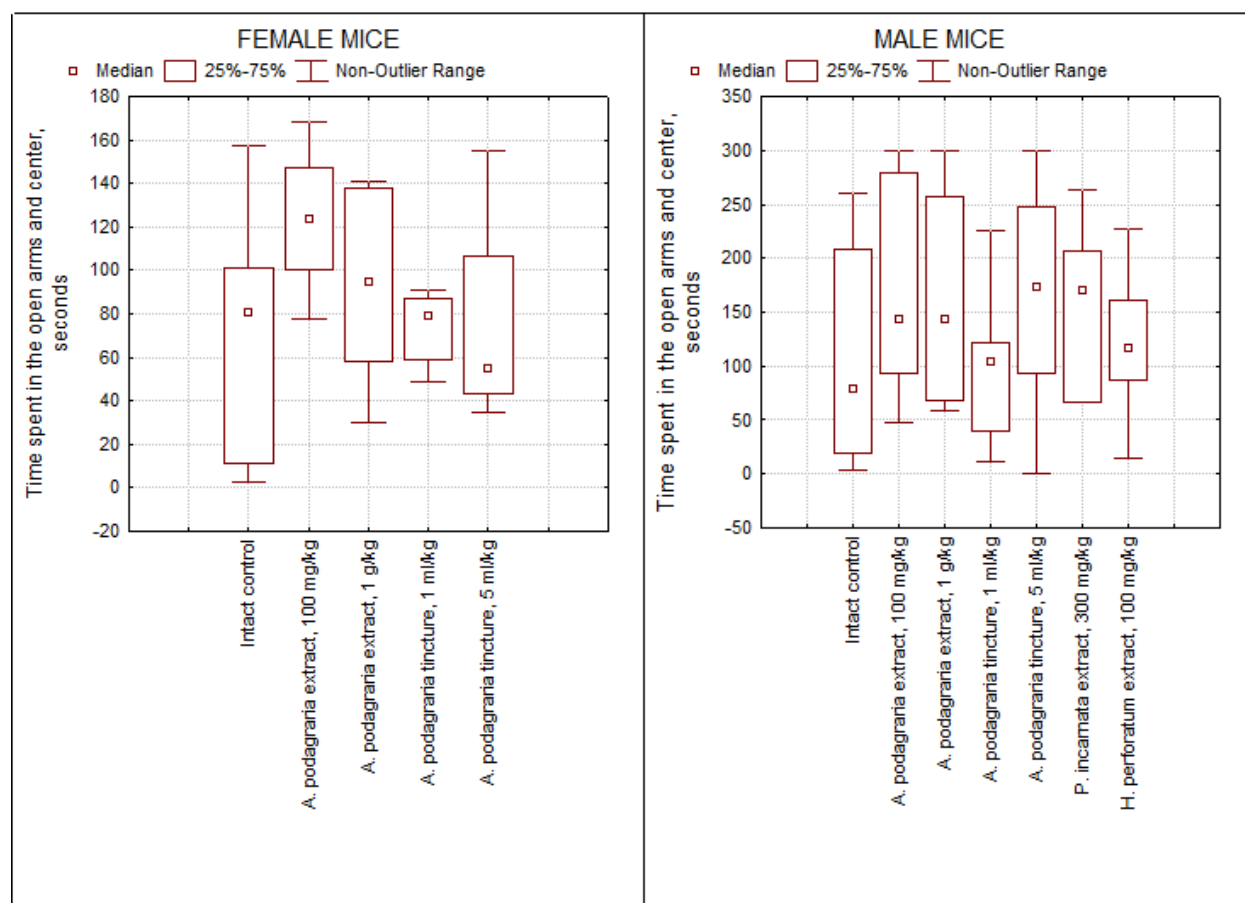
In female mice time spent in the open arm and center was slightly increased only by *A. podagraria* extract at a dose of 100 mg/kg while in male animals this extract at both doses and the tincture at a dose of 5 ml/kg as well as *P. incarnata* extract tended to augment this value (Fig 3). Detailed analysis of time spent in different compartments showed that male mice under the influence of the mentioned preparations stayed longer both in open arms and maze center (except *A. podagraria* extract at a dose of 1 g/kg that influenced only on time spent in open arms), and female mice receiving the extract at lower dose increased the duration of stay in the open arms but not in the center of the maze.

In all groups of animals (except female mice receiving *A. podagraria* extract at a dose of 1 g/kg as well as male mice receiving the tincture at a dose of 5 ml/kg) majority of the mice immediately visited the open arm, in contrast to intact animals (Table 3).

 In female mice there was a tendency towards the reduction in the first stay in the enclosed arm against the background of *A. podagraria* extract at a dose of 100 mg/kg (Fig. 4). In male mice the same change was observed in the animals treated with *A. podagraria* extract at both doses, *H. perforatum* and, particularly, *P. incarnata* extracts (statistically significant differences from the intact control group, excluding *H. perforatum* group). The most marked reduction of the first stay in the enclosed arm was caused by *A. podagraria* extract at both doses and *P. incarnata* extract.

As to the vegetative manifestations in the elevated plus maze, only *P. incarnata* extract significantly reduced the number of fecal boli (this reaction was seen only in one animal and the average rate equalled 0.3, while in the intact control group this value (Q_{50} (Q_{25}–Q_{75})) reached 1 (0,8–1,3), $p<0.05$). *H. perforatum* extract as well as *A. podagraria* tincture did not change the vegetative manifestations of male mice. The effect of *A. podagraria* extract depended on dose: the number of fecal boli equalled 0 (0–1) and 1.5 (1–2) against the background of the extract at low and high dose, respectively. In female mice the same tendency was seen, while *A. podagraria* tincture did not influence on this indicator.

Figure 4. The effect of goutweed and reference drugs on the duration of the first stay in the enclosed arm of the elevated plus maze
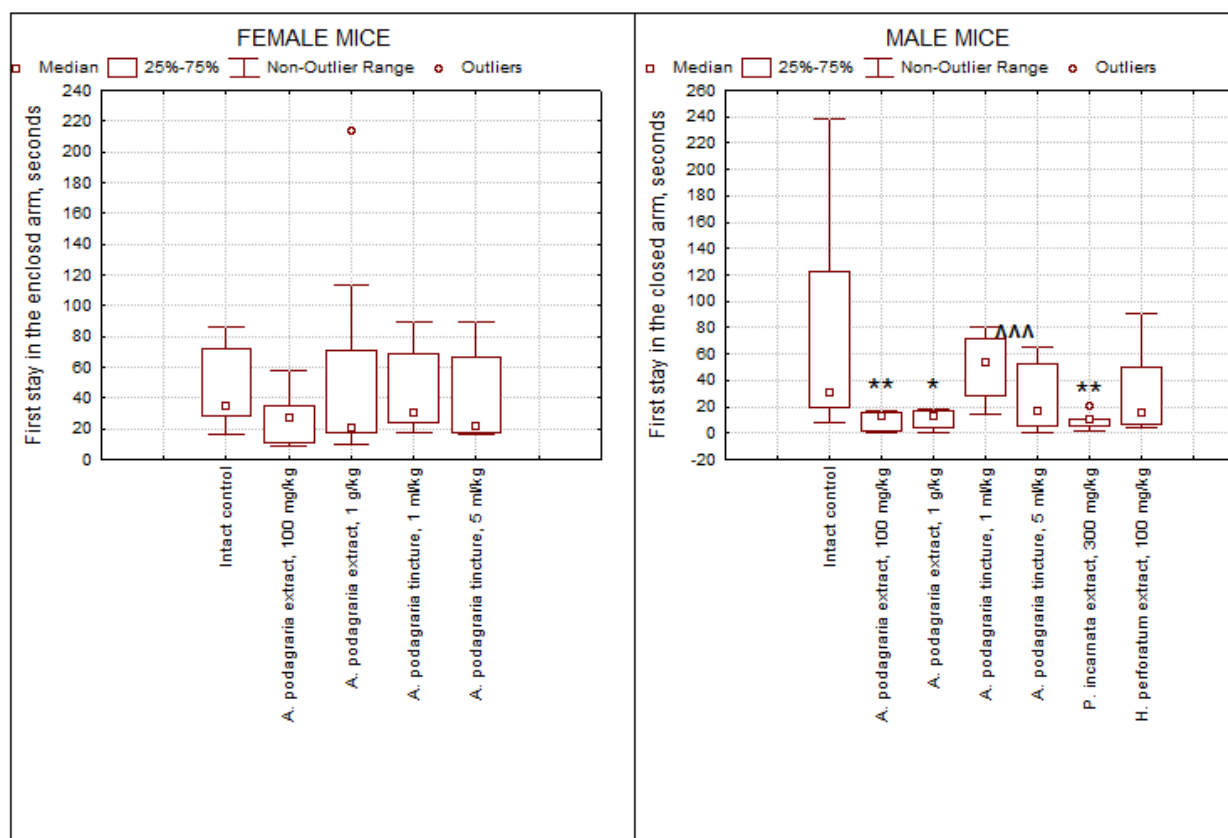
* – Significant at p<0.05 compared with intact control group;
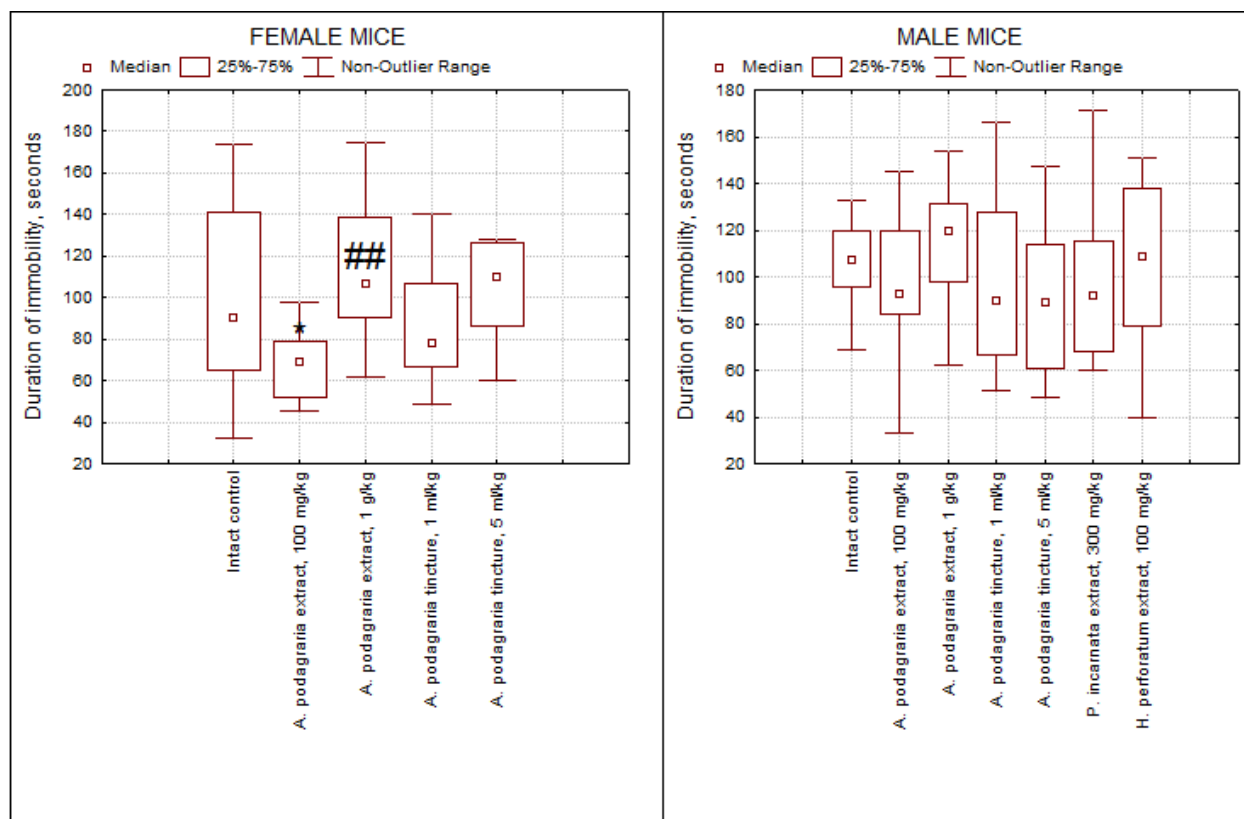*** – Significant at p<0.02 compared with intact control group;*
^^^ – Significant at p<0.001 compared with the group of animals receiving Passiflora extract

In the tail suspension test duration of immobility decreased substantially only in the group of female mice treated with the extract at a dose of 100 mg/kg (p<0.05; Figure 5, 6). Increasing of the extract dose led to the removal of the effect (differences between these groups are statistically significant). *H. perforatum* and *P. incarnata* extracts reduced the number of fecal boli (p<0.05 compared with intact control in both groups) while this value was slightly augmented under the influence of the extract at a low dose (in both sexes) and significantly increased in female mice receiving the extract at the high dose and the tincture at a low dose.

As to the results of the passive avoidance test, in the initial state mice of all groups rapidly entered the dark box and there were no differences in the baseline latency of entry. One day after the training latency of entry into the dark box significantly increased in all groups but this value tended to reduction in animals of both sexes treated with *A. podagraria* extract at a dose of 100 mg/kg and in female mice treated with the tincture at a dose of 1 ml/kg. The number of mice that have acquired the training criterion in these groups decreased (that was statistically significant in female mice receiving the extract, Table 5, 6). No such changes were registered against a background of extract at a dose of 1 g/kg. There were no differences between the groups of female mice in the number of unfinished attempts to entry into the dark box and the latency of the first unfinished attempt. The latter value tended to decrease in male mice under the influence of *A. podagraria* extract at both doses.

A. podagraria preparations did not shown any influence on plasma uric acid level in female mice (data not shown).

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Figure 5. The effect of goutweed and reference drugs on the duration of immobility of mice in the tail suspension test

* – Significant at p<0.05 compared with intact control
\#\# – Significant at p<0.01 compared with the group of animals receiving goutweed extract at a dose of 100 mg/kg

Figure 6. The effect of goutweed and reference drugs on the vegetative manifestations of mice in the tail suspension test
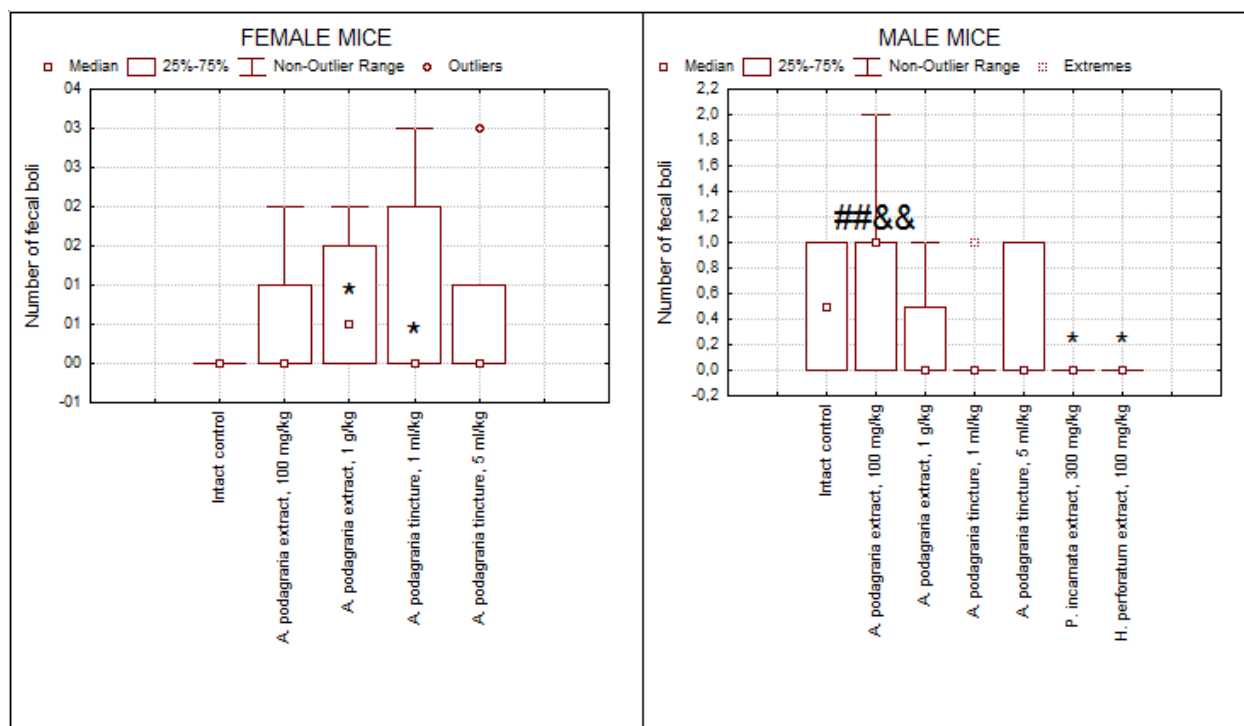
1. * – Significant at p<0.05 compared with intact control group;
2. \#\# – Significant at p<0.02 compared with the group of animals receiving Passiflora extract;
3. && – Significant at p<0.02 compared with the group of animals receiving Hypericum extract.

DISCUSSION

The results of the present study have demonstrated for the first time that *A. podagraria* extract at a dose of 100 mg/kg is able to decrease the level of depression in the tail suspension test in female mice and to reduce the certain signs of anxiety in the elevated plus maze test in animals of both sexes. These effects disappear with the increase in the extract dose. At the same time, locomotion activity in the combined open field test that can be regarded as a sign of sedative action was reduced only in female animals receiving the extract at a dose of 1 g/kg and in male mice treated with the both doses of this extract. *H. perforatum* and *P. incarnata* extracts also decreased several anxiety measures in the elevated plus maze test without any influence on the locomotion activity in the combined open field test. These phenomena are consistent with the data in the literature on the manifestation of herbal drugs' anxiolytic effect in low (sub sedation) doses [35,41,48,49]. Anxiolytic-like effects of *P. incarnata* extract at the dose 300 mg/kg (namely, the statistically significant changes in the latency of entry to the open arm, duration of the first stay in the enclosed arm, vegetative manifestations and number of animals immediately visited the open arm) correspond to the previous findings [49].

On the other hand, the absence of the changes in locomotor activity of female mice treated with the lower dose of the extract may confirm that the reduction in duration of immobility in the tail suspension test was not false positive as suggested in the study [33]. Also it is well known that the antidepressive drugs may reduce locomotor activity [50] and this is consistent with the antidepressive effect of the lower dose of the extract.

According to the data in the literature, several compounds present in *A. podagraria* reduce the level of depression as well as anxiety signs in the experiment. Thus, caffeic acid exerts antidepressive and anxiolytic effects through the indirect modulation of the alpha-1A-adrenoceptor-mediated signal transduction. Under the conditions of the forced swimming stress it attenuates the down-regulation of brain-derived neurotrophic factor transcription. These mechanisms are other than those of drugs that are widely used now [25,51]. In the forced swimming test in mice, caffeic acid significantly reduces the duration of immobility at a dose of 4 mg/kg intraperitoneally [51]. Decaffeinated coffee with high chlorogenic acid content improves mood and behavioural measures in older volunteers [52].

Falcarindiol and ferulic acid exhibit affinity towards 5-HT7 receptors [26], these receptors are considered to be the perspective target of depression treatment [53]. Ferulic acid is a metabolite of caffeic acid [54] that is present in *A. podagraria* so it may be involved into the psychotropic effect of the plant preparations. Falcarindiol unlikely takes part in the activity of *A. podagraria* because it is extracted by ethanol solutions rather than water, and the tincture has shown only the tendency towards anxiolytic action (the increase in the number of mice that immediately visited the open arm after the treatment with both doses in females and with lower dose in males was statistically significant, still clear tendency to increase in the period spent by the light and in latency of entry into the open arms was seen in males receiving the high dose of the tincture).

The decreased level of depression was demonstrated in animals receiving salt substitute that provided surplus intake of potassium and magnesium [27]. *A. podagraria* raw material and preparations are characterized by high potassium content that equals 38–83 mg/g (leaves) and 160 mg/g (dry extract, water extraction) according to data [23], 38 mg/g (leaves) and 77 mg/g (stems) according to data [21]. Still the contribution of these macroelements to the antidepressive properties of the extract is hardly probable in our study. The evidence for this is, firstly, the dose-dependent effect of the extract with the disappearance of activity with the increase of a dose. Secondly, in the study [27] antidepressive action of the salt substitute was shown after 2 months of treatment that is not comparable with the current work terms.

It should be noted that the antidepressive effect of *A. podagraria* extract at a low dose was evident only in female mice. Similar data concerning classical antidepressive drugs are available in the literature: fluoxetine differs in pharmacokinetics and exerts sex-specific effects on neuroplasticity in mice [38], sex-specific differences were registered in the efficacy of fluoxetine and maprotiline in depressed patients [55], that may be attributed both to the differences in pharmacokinetics [38,56] and in pharmacodynamics [36,37]. The peculiarities and mechanisms of sex-specific antidepressive effect of *A. podagraria* extract need further research.

The expected antidepressive effect of *H. perforatum* extract was not registered that is not consistent with the data [34] but corresponds to the results [57] obtained in the forced swimming test using commercially available *H. perforatum* extracts. Also this preparation did not influence significantly on the locomotor activity in the open field. This data also agree with the previous findings concerning the decrease of motor activity in the mice receiving *H. perforatum* extract at a dose of 200 mg/kg intraperitoneally that is higher than antidepressive dose as well as the dose used in our experiments.

As to the results of the elevated plus maze test, the increase in latency of entry into the enclosed arm and in time spent in the open arms is considered to be the main criteria for anxiolytic action [41,44]. According to these markers there was a tendency towards anxiety reduction against a background of *A. podagraria* extract (more expressed in males) and *P. incarnata* extract. This tendency was also accompanied with the increase in the number of arm entries and in quantity of the mice immediately visited the open arm. As to the latter, similar results were shown in the study [41] in mice treated with the preparation obtained from the classical anxiolytic plant common motherworth (*Leonurus cardiaca L., L. quinquelobatus Gilib.*). Latency of entry into the open arm, that is not a classical criterion for the elevated plus maze although may indicate the possibility of anxiety reduction [31], was significantly augmented under the influence of both doses of the extract in both sexes of animals. *A. podagraria* extract also decreased the duration of the first stay in the enclosed arm of the elevated plus maze (especially in male mice), that may also reflect the level of the fear in the animal.

The absence of the clear dose dependence in the effects of *A. podagraria* preparations is consistent with the known data concerning herbal drugs. For instance, the work [58] showed the absence of significant differences between the effects of three doses of *Dioclea grandiflora Mart. ex. Benth* extract in the elevated plus maze and hole-board test (this extract as well as goutweed drugs contains flavonoids).

The tendency towards the reduction of anxiety may be caused by several biologically active substances present in *A. podagraria*. As mentioned above, caffeic acid exerts antidepressive and anxiolytic effects in two different models of stress [25,51]. Caffeic acid at a dose of 1 mg/kg intraperitoneally (but not at higher doses) increases the number of entries and the time spent in the open arms on plus-maze test without affecting locomotion and exploration in the open field test [59]. We have obtained the similar results against the background of *A. podagraria* extract at a dose of 100 mg/kg but not at a dose of 1 g/kg. Chlorogenic acid shows anxiolytic effect coupled with antioxidant activity in the light/dark test, the elevated plus maze, and the free exploratory test. The effect is revealed at a dose of 20 mg/kg intraperitoneally and is associated with the activation of the benzodiazepine receptors [60]. As the average content of hydroxycinnamic acids (in terms of chlorogenic) in *A. podagraria* extract equals 5% [24], the animals received 5 mg/kg of these compounds with the dose of 100 mg/kg. Taking into account pharmacokinetics, this dose is significantly lower than the dose of chlorogenic acid used in the study [60] but close to the dose of caffeic acid investigated by Pereira et al. [59]. So the contribution of hydroxycinnamic acids to the influence of *A. podagraria* on central nervous system is quite possible.

Kaempferol and its glycosides are present in *A. podagraria* [17]. Kaempferol shows an anxiolytic-like activity partly connected with benzodiazepine receptors at doses from 0.02 to 1.0 mg/kg intragastrically [61]. Unlike these results, low affinity for benzodiazepine receptor and no sedative or anxiolytic effects in mice were registered in the study [62].

Complex composition of the investigated *A. podagraria* preparations leads us to expect synergism in their effects including influence on the central nervous system. Under such conditions it is difficult to compare the activity of individual substances and multi-component plant extracts. Further research is needed to determine the active principles of *A. podagraria.*

As to the intensity of vegetative manifestations, in the open field test the number of fecal boli was slightly augmented under the influence of the extract at a dose of 1 g/kg and the tincture at a dose of 1 ml/kg in the animals of both sexes, in the tail suspension test the same tendency was seen only in female mice. In the elevated plus maze test there were no significant changes in this value in females, slight increase in males receiving *A. podagraria* extract at high dose and significant reduction in males receiving *P. incarnata* extract (that may be concerned as the sign of anxiolytic action). The increase in the number of fecal boli in the open field test was not statistically significant and was not accompanied with the other signs of intensified stress in females (such as grooming acts and urinations); the total activity sum was also not changed. So the augmentation in this value can hardly be associated with the intensification of emotional stress response in female mice. Besides being a marker of vegetative maintenance of behavioural reactions, the number of fecal boli reflects gastrointestinal tract functional state and has been used in the work [63] as a marker of laxative action. The direct influence of the *A. podagraria* preparations on the gastrointestinal tract is quite possible but has never been investigated. However, this hypothesis requires further research. On the contrary, in male mice treated with *A. podagraria* preparations as well as *H. perforatum* extract there was an increase in the number of grooming acts, *A. podagraria* extract at both doses significantly augmented the sum of vegetative manifestations in male mice at that. So the mechanism of these changes may be different from that of females and is possible to be mediated through central effects of *A. podagraria* components.

In the passive avoidance test, intact female mice showed slightly better performance than male mice that is generally consistent with the literature data [50]. *A. podagraria* extract at a dose of 100 mg/kg significantly reduced the

number of successfully trained female mice and tended to decrease this value in male mice. On the one hand, such changes usually are considered to be the result of negative influence of the investigated drugs on the memory processes [44,45]. Still the extract at a dose of 100 mg/kg has not caused statistically significant change of the latency of entry into the dark box in the animals of both sexes. On the other hand, it should be noted that such changes are in accordance with the increase in time spent in the open arms of the elevated plus maze. Classical anxiolytics such as diazepam have been shown to worsen the results of the passive avoidance test [45]. It is noteworthy to emphasize the combination of antidepressive effect and worsening of the passive avoidance test results in female mice receiving the extract at a dose of 100 mg/kg. It has been conclusively proven that the antidepressive drugs amitriptylline, maprotiline, fluoxetine impair the acquisition of inhibitory avoidance [50]. Regimens of these drugs administration included course pretreatment regimen similar to our study. Also it is noted that the influence of polymodal psychotropic drugs on the inhibitory avoidance learning can be shadowed by their effects on anxiety and analgesia [50]. This may be related to *A. podagraria* drugs as they showed some influence on anxiety level in the current study and are able to suppress COX-1 [17] that is one of the possible mechanisms of analgesic action.

Besides, it has been shown that arachidonic acid is important for the learning and memory processes, and inhibitors of cyclooxygenase produce amnesia for a passive avoidance task in the chick [64]. As *A. podagraria* preparations decrease inflammation in vivo [15] and suppress COX-1 in vitro [17], this mechanism of influence on the results of the passive avoidance test is possible. Nevertheless, COX-1 suppression is associated with falcarindiol and is more pronounced in alcohol extracts compared with water extracts [17]. This is not account for the water extract influence on memory registered in our study although may explain the influence of the tincture on it. The tendency to the decrease in the number of successfully trained mice was shown at the dose 1 ml/kg but not at the dose of 5 ml/kg. Similar dependence of effect on dose of the tincture was shown for the suppression of carrageenin-induced paw oedema [15]. Still further investigation is needed to clear up *A. podagraria* extract influence on memory.

In contrast to data obtained under the conditions of oxonate-induced hyperuricemia in rats and mice [13,15], *A. podagraria* preparations have not changed uricemia in intact female mice. The same results were obtained in the study [13] on male mice. This indicates that *A. podagraria* components do not cause the disturbance of metabolic reactions in intact organism. So it was not possible to establish the clear interrelation between psychotropic effects and influence on the uric acid metabolism that was expected considering *A. podagraria* preparations pharmacological properties [13–15] and the impact of hypouricemic agents on the CNS functional state [31,33]. As distinct from our previous data [31], there was no relationship between latency of entry into the enclosed arm and the concentration of uric acid in blood plasma. No correlation was observed between uricemia and latency of entry into the open arm, however there was certain interconnection between uricemia and time spent in the open arms. The extract at a dose of 100 mg/kg changed this interconnection direction as $\rho = 0.77$ (p >0.05), against –0.63 (p >0.05) in the intact mice. The coefficients in groups of animals receiving the extract at a dose of 1 g/kg and the tincture at a dose of 1 and 5 ml/kg equalled –0.29; 0.21; –0.95 respectively (in all cases p >0.05).

Thus, the obtained results allowed to prove the absence of severe functional changes of the central nervous system that is principal for the drug (such as goutweed) that is planned to be used in chronic diseases and pathological states. On the other hand, homeostatic reactions of the intact organism might counteract to the realization of pharmacological activity of the herbal drugs. Some favourable changes seen under the influence of A. podagraria preparations complete the pharmacological characteristic of the plant and inspire us to continue the investigations.

CONCLUSION

A. podagraria extract at a dose of 100 mg/kg but not at a dose of 1 g/kg decreases the duration of immobility in the tail suspension test and reduces of the number of successfully trained female mice in the passive avoidance test. The extract at a dose of 100 mg/kg tends to reduce anxiety signs in the elevated plus maze test in the animals of both sexes, in male mice such reduction is also seen under the influence of *A. podagraria* extract at a dose of 1 g/kg and *A. podagraria* tincture at doses of 1 and 5 ml/kg. The reduction in the locomotion activity in the combined open field test appears only in female mice receiving the extract at high dose and, to a lesser extent, in male animals receiving this drug at both doses. Thus, *A. podagraria* tincture does not considerably influence on the central nervous system in intact mice while the extract shows dose-dependent and sex specific antidepressive effect with the worsening of the results of the passive avoidance test and tends to reduce anxiety without significant changes in locomotor activity.

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