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ОРИГИНАЛЬНАЯ СТАТЬЯ

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Antidepressant activity of extracts from the herbs *Astragalus varius* and *Astragalus testiculatus* in the "Tail suspension test"

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The aim. To determine the content of flavonoids in aqueous and aqueous-alcoholic extracts from the herbs of *Astragalus varius* and *Astragalus testiculatus* and to investigate the antidepressant effect of the extracts *in vivo*.

Materials and methods. The objects of the study were dried and ground herbs of *Astragalus varius* S.G. Gmel. and *Astragalus testiculatus* Pall., collected in the Saratov region during the period of mass flowering (May–June 2021). Aqueous (1:10) and aqueous-alcoholic (1:10, extractant 70% ethanol) extracts were obtained from the raw material. The flavonoid content was determined by differential spectrophotometry at an analytical wavelength of 410 nm in quartz cuvettes with *l*=1 on a Shimadzu UV-1800 spectrophotometer (Shimadzu, Japan). The study of antidepressant activity was conducted on male mice weighing 32–38 g and aged 2–3 months using the Tail Suspension Test (TST). The animals received the studied extracts at a dose of 100 mg/kg, and amitriptyline at a dose of 10 mg/kg served as the comparison drug. For data evaluation, the Mann-Whitney U-test and Bonferroni correction (p <0.01) were used. To study the strength of the linear relationship between antidepressant activity and flavonoid content, correlation analysis was used (Spearman correlation coefficient at p <0.05).

Results. The flavonoid content in aqueous and aqueous-alcoholic extracts from the herb of *Astragalus varius* was 2.54 ± 0.04% and 9.31 ± 0.07%, respectively, and in extracts from the herb of *Astragalus testiculatus* — 1.06 ± 0.05% and 10.34 ± 0.05%, respectively. The aqueous-alcoholic extract of *Astragalus testiculatus* demonstrated a pronounced effect reliably similar to amitriptyline (p = 0.01) both after a single oral administration and throughout the entire experimental period (21 days). The aqueous-alcoholic extract of *Astragalus varius* did not show an antidepressant effect after a single administration; however, on days 8, 15, and 21 of administration, a significant (p = 0.01) effect was observed in the animals. Upon administration of the aqueous extract of *Astragalus varius*, an antidepressant effect was observed on days 1, 15, and 21 of the study (p = 0.01); however, the effect was absent on day 8 of the experiment. The aqueous extract of *Astragalus testiculatus*, both after single and chronic oral administration of the extract to animals, showed no activity in the experiment (p >0.01).

Conclusion. Aqueous-alcoholic extracts from the herbs of both species exhibited a more pronounced antidepressant effect compared to aqueous extracts. Correlation analysis established that the identified antidepressant activity is associated with the flavonoid content in the studied extracts.

Keywords: extract; flavonoids; antidepressant activity; *Astragalus varius* S.G. Gmel.; *Astragalus testiculatus* Pall.

Abbreviations: TST — Tail Suspension Test; WHO — World Health Organization; BACs — biologically active compounds; SPh RF XV ed. — State Pharmacopoeia of the Russian Federation XV edition; PhM — pharmacopoeial monograph; SS — standard sample; ROSs — reactive oxygen species; SOD — superoxide dismutase; HPA axis — hypothalamic-pituitary-adrenal axis.

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Антидепрессивная активность экстрактов из травы астрагала изменчивого и астрагала яйцеплодного в тесте «Подвешивание за хвост»

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Цель. Определить содержание флавоноидов в водных и водно-спиртовых экстрактах из травы астрагала изменчивого и астрагала яйцеплодного и *in vivo* исследовать антидепрессивный эффект экстрактов.

Материалы и методы. Объектами исследования были высушенная и измельчённая трава астрагала изменчивого (*Astragalus varius* S.G. Gmel.) и астрагала яйцеплодного (*Astragalus testiculatus* Pall.), заготовленная на территории Саратовской области в период массового цветения (май–июнь 2021 года). Из сырья были получены водные (1:10) и водно-спиртовые (1:10, экстрагент 70% этанол) извлечения. Содержание флавоноидов определяли методом дифференциальной спектрофотометрии при аналитической длине волны 410 нм в кварцевых кюветах *l*=1 на спектрофотометре Shimadzu UV-1800 (Shimadzu, Япония). Исследование антидепрессивной активности проводили на мышах-самцах массой 32–38 г и возрастом 2–3 мес с помощью теста «Подвешивание за хвост/Tail Suspesion Test» (TST). Исследуемые экстракты животные получали в дозе 100 мг/кг, препаратом сравнения служил амитриптилин в дозе 10 мг/кг. Для оценки данных использовали U-критерия Манна-Уитни, поправку Бонферрони (p <0,01). Для изучения тесноты линейной связи между антидепрессивной активностью и содержанием флавоноидов использовали корреляционный анализ (коэффициент корреляции Спирмена при p <0,05).

Результаты. Содержание флавоноидов в водных и водно-спиртовых экстрактах из травы астрагала изменчивого составило 2,54±0,04 и 9,31±0,07% соответственно, а в экстрактах из травы астрагала яйцеплодного — 1,06±0,05 и 10,34±0,05% соответственно. Водно-спиртовой экстракт астрагала яйцеплодного демонстрировал достоверно аналогичный амитриптилину выраженный эффект (p=0,01) как после однократного перорального введения, так и на протяжении всего периода эксперимента (21 день). Водно-спиртовой экстракт астрагала изменчивого не проявлял антидепрессивный эффект после однократного введения, однако на 8, 15 и 21 сутки приёма наблюдалось достоверное (p=0,01) проявление эффекта у животных. При введении водного экстракта астрагала изменчивого наблюдался антидепрессивный эффект на 1, 15 и 21 сутки исследования (p=0,01), однако эффект отсутствовал на 8 день эксперимента. Водный экстракт астрагала яйцеплодного, как после однократного, так и после хронического перорального введения экстракта животным не показал активности в эксперименте (p >0,01).

Заключение. Водно-спиртовые экстракты из травы обоих видов обладали более выраженным антидепрессивным эффектом по сравнению с водными. С помощью корреляционного анализа установлено, что выявленная антидепрессивная активность связана с содержанием флавоноидов в исследуемых извлечениях.

Ключевые слова: экстракт; флавоноиды; антидепрессивная активность; *Astragalus varius* S.G. Gmel.; *Astragalus testiculatus* Pall.

Список сокращений: TST — тест «Подвешивание за хвост»; ВОЗ — Всемирная организация здравоохранения; БАС — биологически активные соединения; ГФ РФ XV изд. — Государственная фармакопея Российской Федерации XV издания; ФС — фармакопейная статья; СО — стандартный образец; АФК — активные формы кислорода; СОД — супероксиддисмутаза; ГГНС — гипоталамо-гипофизарно-надпочечниковая система.

INTRODUCTION

Globally, approximately 350 million people suffer from depression, and Russia ranks 4th in the world in terms of disease prevalence (38 % of the population across different age groups), according to an assessment by the World Health Organization (WHO) as of 2021. Synthetic antidepressants are used for the therapy of depressive disorders, but their use

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often leads to the development of not only therapeutic but also undesirable effects [1–3]. It has been found that pharmacotherapy with antidepressants is ineffective in one-third of patients due to emerging side effects [4–6].

Phytopreparations can be used for the treatment of mild to moderate depressive states [2, 7]. Medicines of St. John's Wort (*Hypericum perforatum* L.) have shown the greatest efficacy in treating mild depression in adults [8, 9]. Given that biologically active components isolated from St. John's Wort herb are well-tolerated, drugs based on them can be recommended for long-term use, including for maintenance therapy [8]. Antidepressant activity has also been identified in other plants, for example, in *Crataegus submollis* Sarg. [9], *Anisum vulgare* Goerth. [10], *Rhodiola rosea* L. [11], *Magnolia grandiflora* L. [12], and others.

One of the promising sources of biologically active compounds (BACs) are plants of the genus *Astragal*, which comprises over 3000 species. The most studied is *Astragalus membranaceus* (Fisch.) Bunge. Drugs from the roots of *Astragalus membranaceus* are included in the State Pharmacopoeia of China and are used for various diseases as immunomodulatory, cardioprotective, and antitumor agents [13–16].

Individual components and complex extracts obtained from some *Astragalus* species exhibit neuroprotective activity [17]. The neuroprotective effect of a methanolic extract from the shoots of *Astragalus spinosus* against bisphenol A-induced anxiety and depression in a rat model of postnatal Schizophrenia is known [18]. The combination of anxiolytic and potential antidepressant effects of *Astragalus membranaceous var. A. mongholicus* was similar to the action of a benzodiazepine derivative—alprazolam, but demonstrated some differences from alprazolam, including the absence of sedative effects and amnesia [19]. A comprehensive assessment of animal behavior parameters in the "Suck Test" showed that administration of an extract from the herb *Astragalus vulpinus* Willd under conditions of informational stress had a corrective effect on the psychoemotional status, which manifested in the activation of the orienting-exploratory component of behavior, as well as in the elimination of anxiety-depressive behavioral disorders in white rats [20].

Despite the active study of plants of the genus *Astragal*, scientific data is currently insufficient. Furthermore, the lack of data on the chemical composition and biological activity of most representatives of the genus provides a basis for their research and defines the relevance of this direction.

Astragalus varius S.G. Gmel. and *Astragalus testiculatus* Pall. are widely distributed in the Volga region. Extracts from *Astragalus varius* and *Astragalus testiculatus* have shown pronounced antimicrobial activity against *S. aureus, E. coli, P. aeroginosa* [21] and antioxidant activity based on the level of inhibition of adrenaline hydrochloride auto-oxidation in an alkaline medium [22], as well as low toxicity in in vivo experiments [23]. The diuretic effect of an infusion of *Astragalus varius* herb in a 4-hour experiment exceeded the diuretic effect of furosemide at a threshold dose of 1 mg/kg, and in a 24-hour experiment was slightly lower than that of hydrochlorothiazide at a medium therapeutic dose of 20 mg/kg [24].

Phytochemical studies have revealed the presence of flavonoid compounds with potential antidepressant activity. Thus, isoquercitrin, rutin, hyperoside, narcissin, cynaroside, and astragalin were found in the herb of *Astragalus varius*, and rutin, cynaroside, and astragalin were found in the herb of *Astragalus testiculatus* [25]. It was previously established that the antidepressant effect of St. John's Wort herb-based medicines is primarily associated with the action of flavonoids—hyperoside and bisapigenin [9]. Additionally, antidepressant properties of another flavonoid glycoside—astragalin—are known [26]. The antidepressant activity of extracts from the herbs *Astragalus varius* and *Astragalus testiculatus* has not been previously studied, which is of interest for investigation.

THE AIM. To determine the flavonoid content and investigate the antidepressant activity of aqueous and aqueous-alcoholic extracts from the herbs *Astragalus varius* and *Astragalus testiculatus*.

MATERIALS AND METHODS

Experimental design

The experimental design is presented in Figure 1.

Preparation of active substances

For the study of biological activity, aqueous and aqueous-alcoholic extracts from the herb of two species of *Astragalus* were selected: *Astragalus varius (A. varius* S.G. Gmel.) and *Astragalus testiculatus* (*A. testiculatus* Pall.).

The plant raw material was collected during the period of mass flowering in the Saratov region in May–June 2021. Drying was carried out by air-shade method to a residual moisture content of no more than 12 %.

Aqueous extracts (1:10) were prepared to the method described in GPhM.1.4.1.0018 "Infusions and Decoctions"[1] of the State Pharmacopoeia of the Russian

[1] GPhM.1.4.1.0018 "Infusions and decoctions". State Pharmacopoeia of the Russian Federation XV edition. Available from: https://pharmacopoeia.regmed.ru/pharmacopoeia/izdanie-15/1/1-4/1-4-1-lekarstvennye-formy/nastoi-i-otvary/. Russian

Federation, XV edition (SPh RF XV ed.); aqueous-alcoholic extracts (70 % ethanol) were obtained by maceration for 6 days in a raw material-to-extractant ratio of 1 : 10. The extracts were filtered, concentrated to the state of a thick extract, after which the residue was dried in a drying oven at 40 °C to constant weight.

To assess antidepressant activity, the obtained dry residues were dissolved in distilled water to obtain a concentration of 100 mg/mL.

Phytochemical analysis

To assess the content of the sum of flavonoids, the method of differential spectrophotometry calculated as rutin [27] was used. For this, 0.1 g of dry residue was dissolved in 10 mL of 70 % ethyl alcohol in a 25 mL volumetric flask (solution A). Then, 1 mL of solution A was placed in a 25 mL volumetric flask, 5 mL of 5% aluminum (III) chloride solution and 0.5 mL of acetic acid solution were added. The analysis was performed after 30 min on a Shimadzu UV-1800 spectrophotometer (Shimadzu, Japan) in quartz cuvettes with a layer thickness of 10 mm at an analytical wavelength of 410 nm relative to a reference solution (1 mL of solution A without the addition of a complexing agent). Under similar conditions, the optical density of a rutin standard sample of 0.05 % was determined.

Preparation of Rutin Solution. Approximately 0.05 g (exact weight) of rutin standard (≥ 95 %, No. 89270, lot No. 66853802, PhytoLab, Germany) was placed in a 100 mL volumetric flask, 85 mL of 70 % alcohol was added, and heated in a water bath until completely dissolved. Then, it was cooled, the volume of the solution was brought to the mark with the same solvent, and mixed. The shelf life of the solution is 30 days when stored in a well-sealed container, in a cool, light-protected place.

The content of flavonoids calculated as rutin was determined by the formula:

$$X(\%) = \frac{A \times 25 \times m_0 \times 100}{A_0 \times m \times (100 - W)},$$

where A is the optical density of the test solution; A_0 is the optical density of the rutin standard solution; m is the mass of the extract, g; m_0 is the mass of the rutin standard, g; W is the loss on drying, %.

Experimental animals

Antidepressant activity was determined on 36 outbred male mice housed in the vivarium of the common use center for experimental oncology of the Saratov State Medical University named after V.I. Razumovsky, weighing 32–38 g and aged 2–3 months. The animals were kept under standard vivarium conditions with a 12-hour light cycle, constant temperature and humidity, with free access to food and water.

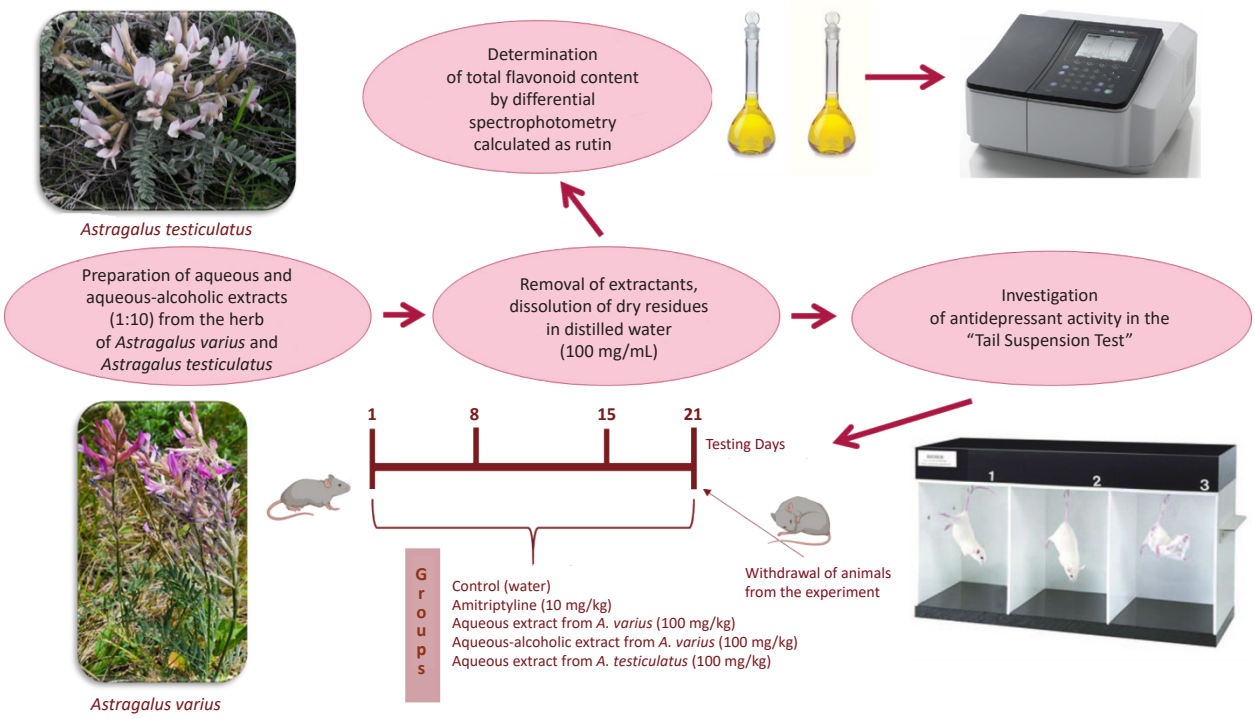

Figure 1 — Experimental design.

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The design of the experimental study was approved by the Local Ethics Committee of the Saratov State Medical University named after V.I. Razumovsky (Protocol No. 4 dated Feb 01, 2022).

The study groups are presented in Table 1 (each group received a daily intragastric administration of the test substance solution using a probe). Amitriptyline was chosen as the comparison drug at a therapeutic dose of 10 mg/kg (average 0.35 mg per day; OZON PHARM LLC, expiry date 12.2023) [28], and the test extracts were used at a dose of 100 mg/kg (average 3.5 mg per day). The choice of dose for the test extracts is due to the fact that antimicrobial and diuretic activity were previously found in extracts from the herb of *Astragalus varius* and *Astragalus testiculatus* at the indicated dose [25, 28]. We previously identified the effect of an aqueous extract from the herb of *Astragalus membranaceus* at a dose of 100 mg/kg on the cognitive functions of rats in the "eight-arm radial maze" test, in which the infusion of *Astragalus membranaceus* caused activation of working and long-term spatial memory [29].

Antidepressant activity

To assess the antidepressant activity of the test substances, the "Tail Suspension Test" (TST; OpenScience, Russia) was used, which was conducted on days 1, 8, 15, and 21 of the experiment. During the test, mice were suspended by their tails on sticky tape; the testing duration was 3 minutes.

To assess behavior, the following parameters were recorded: total time of activity maintenance (s), total duration of immobility (s), latent period of the first episode of immobility (s) [30].

Laboratory animals were euthanized at the end of the 21st day by overdose of anesthetic drugs — an intraperitoneal combination of Zoletil (tiletamine 250 mg and zolazepam 250 mg; (Virbac, France) and Xylanit (xylazine; Nita-Pharm, Russia) at a dose of 0.1 mg/kg.

Statistical analysis

Statistical analysis of the results was carried out using the Statistica 10.0 software package (StatSoft Inc., USA). Sample normality of distribution was checked using the Shapiro-Wilk test. During statistical processing of the study results, the distribution of trait values differed from normal, therefore, the Mann-Whitney U-test was used to evaluate the data with recalculation of the significance level (Bonferroni correction was applied) considering multiple comparisons (5)— $p < 0.01$. For each indicator, the median (Me) and interquartile range [Q1; Q3] were calculated. To study the strength of the linear relationship between indicators, correlation analysis was used—the Spearman correlation coefficient was calculated. With positive r values, a direct relationship was identified; with negative values, an inverse relationship; with 0, no relationship. The strength of the relationship was assessed by the values of the r coefficient (from 0 to 0.3—very weak, from 0.3 to 0.5—moderate, 0.5–0.7—medium, 0.7–0.9—high, 0.9–1.0—very high). Results were considered significant at $p < 0.05$.

RESULTS

Phytochemical analysis

The study of the electronic spectra of aqueous and aqueous-alcoholic extracts from the herb of *Astragalus* species (Fig. 1) showed the presence of two absorption bands with maxima at 270 nm and 330 nm, characteristic of flavonoids. Upon addition of 5 % aluminum (III) chloride solution, a bathochromic shift to the long-wavelength region of approximately 70 nm was observed, and under differential spectrophotometry conditions, the maximum absorption of the long-wavelength band was recorded in the range of 402–409 nm (Fig. 2). Rutin (λmax = 410 ± 0.2 nm) was chosen as the standard sample.

The results of determining the flavonoid content calculated as rutin are presented in Table 2.

It was found that the flavonoid content in the aqueous-alcoholic extract from *Astragalus ovatus* herb (10.34 ± 0.05 %) is higher than in the aqueous-alcoholic extract from Astragalus varius herb (9.31 ± 0.07 %). The sum of flavonoids in the aqueous extract of *Astragalus ovatus* (1.06 ± 0.05 %) is 2 times less than in the aqueous extract of *Astragalus varius* (2.54 ± 0.04 %).

Antidepressant activity

The results of the study of antidepressant activity over 21 days of the experiment are presented in Tables 3–6.

In animals in the group receiving amitriptyline (10 mg/kg) throughout the 21-day experiment (Group 2), a pronounced antidepressant effect was observed. The time of activity maintenance was longer than in mice in the control group: day 1 by 98.5% ($p = 0.02$) (see Table 3), day 8 by 105.9% ($p = 0.004$) (see Table 4), day 15 by 91.6 % ($p = 0.004$) (see Table 5), day 21 by 139.1 % ($p = 0.011$) (see Table 6), and the duration of immobilization was shorter: day 1 by 59.4 % ($p = 0.025$) (see Table 3), day 8 by 93.2 % ($p = 0.004$) (see Table 4), day 15 by 78.5 % ($p = 0.004$) (see Table 5), day 21 by 80.6 % ($p = 0.01$) (see Table 6). The latent period of the first episode of immobilization did not show statistically significant differences from the control group ($p > 0.01$).

Table 1 — Experimental animal groups in the "Tail Suspension Test"

Group No.	1	2	3	4	5	6
Name	Control (Water)	Amitriptyline	Aqueous extract of *A. varius*	Alcoholic extract of *A. varius*	Aqueous extract of *A. testiculatus*	Alcoholic extract of *A. testiculatus*
Dose, mg/kg	–	10	100	100	100	100
Dose per day, mg	–	0.35	3.5	3.5	3.5	3.5

Table 2 — Results of determining the flavonoid content in extracts from *Astragalus varius* and *Astragalus ovatus* herbs in % (P=0.95; *n*=3)

Extract name	Average value, $\overline{X}$ (%)	Variance, S^2	Standard deviation (S_x), *SD*	Standard deviation of the mean result, $S_{\overline{x}}$	Relative standard deviation, RSD (%)	Deviation from the average value, $\Delta\overline{X}$ (%)	Relative error, ε (%)
Aqueous *Astragalus varius*	2.54	0.001233333	0.03512	0.02028	1.384	0.04	3.44
Aqueous-alcoholic *Astragalus varius*	9.31	0.001600000	0.04000	0.02309	0.430	0.07	1.07
Aqueous *Astragalus ovatus*	1.06	0.000400000	0.02000	0.01155	1.887	0.05	4.68
Aqueous-alcoholic *Astragalus ovatus*	10.34	0.000400000	0.02000	0.01155	0.193	0.05	0.48

Table 3 — "Tail Suspension Test", Day 1 of the experiment

Group of animals	Indicator								
	Total time of activity maintenance, sec			Total duration of inactivity, sec			Latent period of the first episode of immobilization, sec		
	Me [Q1; Q3]	p_1	p_2	Me [Q1; Q3]	p_1	p_2	Me [Q1; Q3]	p_1	p_2
Control	68.00 [54.75; 75.75]	–	–	112.00 [97.75; 119.75]	–	–	7.00 [4.75; 13.75]	–	–
Amitriptyline, 10 mg/kg	135.00 [103.00; 167.25]	0.02	–	45.50 [14.25; 75.75]	0.02	–	46.00 [26.00; 48.50]	0.13	–
Aqueous extract of *Astragalus varius*, 100 mg/kg	113.50 [109.25; 122.25]	0.01	0.33	66.50 [47.25; 70.25]	0.01	0.66	32.50 [16.25; 43.00]	0.13	0.52
Aqueous-alcoholic extract of *Astragalus varius*, 100 mg/kg	86.00 [66.00; 101.00]	0.20	0.19	94.00 [53.00; 102.00]	0.20	0.20	25.50 [0.00; 59.25]	0.83	0.66
Aqueous extract of *Astragalus ovatus*, 100 mg/kg	99.50 [74.25; 114.75]	0.08	0.28	80.50 [47.75; 93.25]	0.08	0.39	16.50 [8.00; 25.00]	0.39	0.14
Aqueous-alcoholic extract of *Astragalus ovatus*, 100 mg/kg	132.00 [113.25; 146.00]	0.01	0.83	48.00 [24.00; 60.25]	0.01	1.0	65.50 [26.00; 92.75]	0.03	0.29

Note: significance of differences by Mann-Whitney criterion (at $p < 0.01$); "p_1" — difference from control; "p_2" — difference from amitriptyline.

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Table 4 — "Tail Suspension Test", Day 8 of the experiment

Group of animals	Indicator								
	Total time of activity maintenance, sec			Total duration of inactivity, sec			Latent period of the first episode of immobilization, sec		
	Me [Q1; Q3]	p_1	p_2	Me [Q1; Q3]	p_1	p_2	Me [Q1; Q3]	p_1	p_2
Control	84.50 [76.25; 89.00]	–	–	95.50 [89.00; 99.25]	–	–	16.00 [9.50; 21.50]	–	–
Amitriptyline, 10 mg/kg	174.50 [136.75; 178.75]	0.003	–	6.50 [0.75; 32.00]	0.004	–	44.00 [27.00; 137.50]	0.14	–
Aqueous extract of *Astragalus varius*, 100 mg/kg	98.50 [78.50; 120.50]	0.45	0.02	100.00 [73.75; 177.25]	0.59	0.01	33.50 [21.25; 45.50]	0.1	0.45
Aqueous-alcoholic extract of *Astragalus varius*, 100 mg/kg	136.50 [130.00; 144.25]	0.01	0.13	43.50 [27.25; 48.00]	0.01	0.16	11.50 [2.50; 27.25]	0.45	0.29
Aqueous extract of *Astragalus ovatus*, 100 mg/kg	117.00 [82.00; 128.50]	0.14	0.03	63.00 [44.50; 80.00]	0.13	0.03	15.50 [6.00; 22.00]	0.75	0.08
Aqueous-alcoholic extract of *Astragalus ovatus*, 100 mg/kg	144.00 [112.50; 157.75]	0.01	0.13	36.00 [18.75; 54.50]	0.01	0.14	58.00 [19.75; 72.75]	0.16	0.83

Note: significance of differences by Mann-Whitney criterion (at $p < 0.01$); "p_1" — difference from control; "p_2" — difference from amitriptyline.

Table 5 — "Tail Suspension Test", Day 15 of the experiment

Group of animals	Indicator								
	Total time of activity maintenance, sec			Total duration of inactivity, sec			Latent period of the first episode of immobilization, sec		
	Me [Q1; Q3]	p_1	p_2	Me [Q1; Q3]	p_1	p_2	Me [Q1; Q3]	p_1	p_2
Control	77.50 [72.50; 93.00]	–	–	102.50 [81.75; 104.50]	–	–	16.50 [12.25; 19.25]	–	–
Amitriptyline, 10 mg/kg	148.50 [108.50; 158.50]	0.004	–	22.00 [15.00; 39.50]	0.003	–	23.50 [2.25; 40.75]	1.00	–
Aqueous extract of *Astragalus varius*, 100 mg/kg	127.50 [109.50; 144.00]	0.01	0.52	52.50 [30.00; 67.50]	0.01	0.08	38.50 [14.75; 33.75]	0.14	0.68
Aqueous-alcoholic extract of *Astragalus varius*, 100 mg/kg	122.50 [101.50; 128.75]	0.01	0.27	57.50 [41.75; 65.50]	0.01	0.06	19.00 [2.75; 37.50]	0.83	0.92
Aqueous extract of *Astragalus ovatus*, 100 mg/kg	85.50 [64.00; 109.25]	1.00	0.05	94.50 [66.25; 116.00]	1.00	0.01	5.50 [0.00; 13.25]	0.16	0.28
Aqueous-alcoholic extract of *Astragalus ovatus*, 100 mg/kg	131.00 [119.50; 133.50]	0.01	0.39	49.00 [43.50; 53.50]	0.01	0.09	36.50 [24.75; 50.75]	0.01	0.39

Note: significance of differences by Mann-Whitney criterion (at $p < 0.01$); "p_1" — difference from control; "p_2" — difference from amitriptyline.

Table 6 — Tail Suspension Test, Day 21 of the Experiment

Group of animals	Indicator								
	Total time of activity maintenance, sec			Total duration of inactivity, sec			Latent period of the first episode of immobilization, sec		
	Me [Q1; Q3]	p_1	p_2	Me [Q1; Q3]	p_1	p_2	Me [Q1; Q3]	p_1	p_2
Control	66.50 [51.25; 90.75]	–	–	113.50 [81.25; 124.25]	–	–	11.00 [6.50; 13.25]	–	–
Amitriptyline, 10 mg/kg	159.00 [122.50; 174.00]	0.01	–	22.00 [3.50; 35.00]	0.013	–	19.00 [0.00; 48.00]	0.71	–
Aqueous extract of *Astragalus varius*, 100 mg/kg	109.50 [107.00; 118.50]	0.01	0.14	70.5 [48.50; 73.00]	0.01	0.14	30.00 [18.00; 37.50]	0.03	0.80
Aqueous-alcoholic extract of *Astragalus varius*, 100 mg/kg	136.50 [126.00; 147.75]	0.01	0.27	43.50 [24.75; 52.00]	0.01	0.33	16.00 [0.00; 39.50]	0.91	1.00
Aqueous extract of *Astragalus ovatus*, 100 mg/kg	91.00 [88.75; 101.50]	0.19	0.03	89.00 [57.50; 89.75]	0.19	0.03	5.00 [2.75; 11.50]	0.19	0.80
Aqueous extract of *Astragalus ovatus*, 100 mg/kg	143.50 [122.75; 157.75]	0.01	0.46	36.50 [12.75; 49.75]	0.01	0.46	48.50 [26.25; 88.50]	0.02	0.14

Note: significance of differences by Mann-Whitney criterion (at $p < 0.01$); "p_1" — difference from control; "p_2" — difference from amitriptyline.

Table 7 — Correlation of the content of flavonoids and the antidepressant activity of aqueous and aqueous-alcoholic extracts of the *Astragalus varius* and *Astragalus ovatus* herbs

Indicator	Day of experiment	Group of animals			
		Aqueous extract of Astragalus varius, 100 mg/kg	Aqueous-alcoholic extract of *Astragalus varius*, 100 mg/kg	Aqueous extract of *Astragalus ovatus*, 100 mg/kg	Aqueous extract of *Astragalus ovatus*, 100 mg/kg
Total time of activity maintenance, sec	1	$r = 0.5$*	$r = -0.5$*	$r = -0.5$*	$r = -0.5$*
	8	$r = -1$*	$r = 0.6$*	$r = -0.5$*	$r = 0.5$*
	15	$r = -0.5$*	$r = 0.5$*	$r = -0.5$*	$r = 0.5$*
	21	$r = -0.9$*	$r = 0.6$*	$r = -0.5$*	$r = 0.5$*
Total duration of inactivity, sec	1	$r = -0.5$*	$r = 0.5$*	$r = 0.5$*	$r = 0.5$*
	8	$r = 1.0$*	$r = 0.6$*	$r = 0.5$*	$r = 0.5$*
	15	$r = 0.5$*	$r = 0.5$*	$r = 0.5$*	$r = 0.5$*
	21	$r = 0.9$*	$r = 0.6$*	$r = 0.5$*	$r = 0.5$*
Latent period of the first episode of immobilization, sec	1	$r = 0.5$	$r = 0$	$r = -0.5$	$r = -0.5$
	8	$r = -0.5$	$r = -0.3$	$r = -1$	$r = -0.5$
	15	$r = -0.5$	$r = -1$	$r = -1$	$r = -0.5$
	21	$r = -0.5$*	$r = 0.3$*	$r = 0.5$*	$r = 0.5$*

Note: * — $p < 0.05$ (Spearman correlation coefficient).

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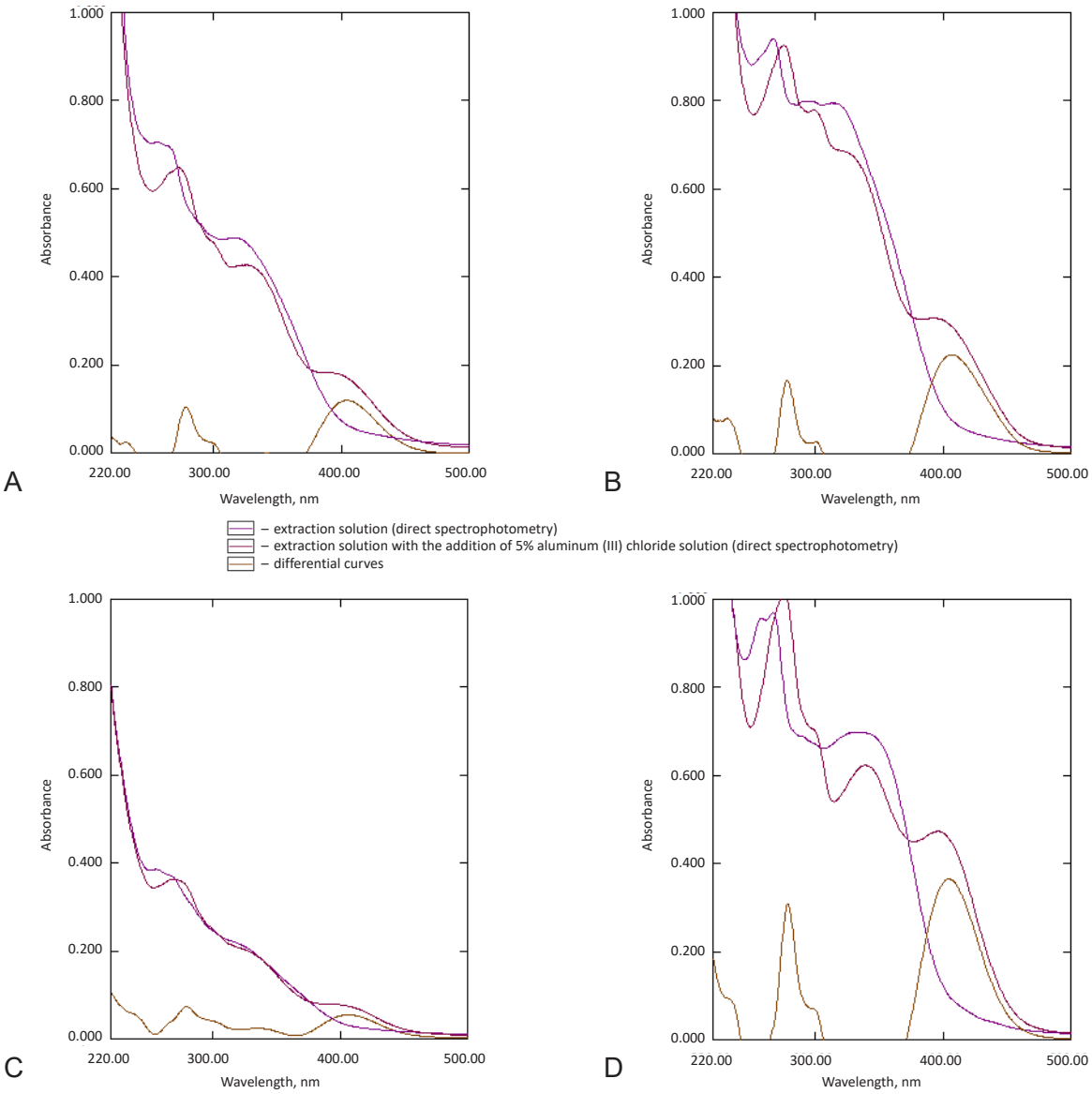

Figure 2 — Electronic spectra of extracts from the herb.
Note: A — *Astragalus ovatus*, aqueous; B — *Astragalus ovatus*, aqueous-alcoholic; C — *Astragalus varius*, aqueous; D — *Astragalus varius*, aqueous-alcoholic.

In mice of the 3rd experimental group, receiving aqueous extract of *Astragalus varius* (100 mg/kg), an antidepressant effect was observed with chronic administration of the extract. Thus, on days 15 and 21, the time of activity maintenance (p = 0.01) and the duration of the immobilization period (p = 0.01) were comparable to the values of the 2nd experimental group receiving amitriptyline (see Tables 5, 6). On day 1 of the experiment, a statistically significant antidepressant effect was also detected (p = 0.01) (see Table 3); however, on day 8 of the experiment, the effect was absent (p > 0.01), which requires further research (see Table 4). The latent period of the first episode of immobilization also did not show statistically significant differences from the control group and the group receiving amitriptyline (p > 0.01).

In animals of the 4th experimental group, receiving aqueous-alcoholic extract of *Astragalus varius* (100 mg/kg), an antidepressant effect was detected after a week of extract administration. On days 8, 15, and 21 of the experiment, the time of activity maintenance (p = 0.01) and the total duration of inactivity (p = 0.01) were comparable to the group receiving amitriptyline (Tables 4–6). On day 1 of testing, the indicators did not statistically differ from the values in the control group (p = 0.02) (see Table 3). The latent period of the first episode of immobilization also did not show statistically

significant differences from the control group and the group receiving amitriptyline ($p > 0.01$).

In animals of the 5th experimental group, receiving aqueous extract of *Astragalus ovatus* (100 mg/kg), an antidepressant effect was absent throughout the experiment ($p > 0.01$).

In animals of the 6th experimental group, receiving alcoholic extract of *Astragalus ovatus* (100 mg/kg), a pronounced antidepressant effect was detected both after a single administration and throughout the entire experiment. From day 1 of the experiment, the time of activity maintenance and the duration of the immobilization period were comparable to the indicators of animals in the control group. On day 1, the time of activity maintenance was higher than in the control group by 49.5% ($p = 0.01$), and inactivity was lower by 2.3 times ($p = 0.01$) (see Table 3). On day 8, activity exceeded the control by 41.7% ($p = 0.01$), and the time of inactivity decreased by 62.2 % ($p = 0.01$) (see Table 4). On day 15, animals maintained activity by 40.9 % ($p = 0.011$) compared to the control (see Table 5). On day 21, the activity of the experimental group was 2.2 times higher than that of the control group ($p = 0.01$), and the indicators of inactivity time were lower by 67.9 % ($p = 0.01$) (see Table 6). The latent period of the first episode of immobilization on days 1, 8, and 21 did not show statistically significant differences from the control group and the group receiving amitriptyline ($p > 0.01$) (see Tables 3, 4, 6), and on day 15 it was higher than in the control group ($p = 0.01$) (see Table 5).

Correlation analysis was performed to determine the relationship between flavonoid content and the antidepressant activity of aqueous and aqueous-alcoholic extracts. For the aqueous extract of *Astragalus varius* herb, a significant moderate positive correlation was observed between flavonoid content and total activity maintenance time on day 1 of the experiment ($r = 0.5$), while no correlation was found on days 8, 15, and 21 of the experiment. The correlation between flavonoid content and total inactivity duration was very high on day 8 ($r = 1$), moderate on day 15 ($r = 0.5$), and high on day 21 (Table 7).

For the aqueous extract of *Astragalus ovatus* herb, there was no correlation between total flavonoid content and total activity maintenance time; however, a significant moderate correlation was found between total flavonoid content and total inactivity duration on days 1, 8, 15, and 21 of the experiment ($r = 0.5$).

For the aqueous-alcoholic extracts of *Astragalus varius* and *Astragalus ovatus* herbs, a direct significant correlation was observed between flavonoid content and total activity maintenance time on days 8, 15, and 21 of the experiment, and a direct significant moderate correlation between total flavonoid content and total inactivity duration on days 1, 8, 15, and 21 of the experiment (see Table 7).

The correlation between flavonoid content and the latency period of the first episode of immobilization was insignificant for almost all extracts (see Table 7).

Analysis of the obtained data showed that alcoholic extracts from the herbs of both “Astragalus” species exhibited a more pronounced antidepressant effect compared to aqueous extracts, which is attributed to the flavonoid content in the extracts: the sum of flavonoids in alcoholic extracts is significantly higher than in aqueous extracts (see Table 2), which is confirmed by the correlation relationships. The aqueous-alcoholic extract of *Astragalus ovatus* demonstrated a significantly similar pronounced effect to amitriptyline ($p = 0.01$) after both single oral administration and throughout the entire experimental period (21 days). The alcoholic extract of *Astragalus varius* did not show an antidepressant effect after single administration; however, after 1 week of administration, a significant ($p = 0.01$) effect was observed on days 15 and 21 in animals.

After single administration of the aqueous extract of *Astragalus varius*, an antidepressant effect was observed, which was nullified after a week of extract administration. When the extract was administered to animals for 2 weeks, a significant ($p = 0.01$) activity was observed. The aqueous extract of *Astragalus ovatus*, after both single and chronic oral administration to animals, showed no activity in the experiment.

Thus, the most pronounced antidepressant properties were found in the aqueous-alcoholic extract of *Astragalus ovatus*, the least in the aqueous extract of *Astragalus varius*, and they were entirely absent in the aqueous extract of *Astragalus ovatus*.

DISCUSSION

The data on the study of the antidepressant activity of extracts from *Astragalus varius* and *Astragalus ovatus* herbs have been obtained by us for the first time. Comparison of the activity of extracts from *Astragalus varius* and *Astragalus ovatus* herbs with extracts from other “Astragalus” species is difficult due to differences in experimental conditions. Under similar conditions, we have studied extracts from *Astragalus*

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membranaceus herb, which is used in traditional medicine for its diuretic and hypotensive effects. Antidepressant properties of aqueous and aqueous-alcoholic extracts from *Astragalus membranaceus* herb were not detected [31], which may be due to the absence or low content of BACs capable of affecting the central nervous system.

It has been established that stress exposure contributes to the development of anxiety-depressive disorders, which require correction. For these purposes, medicinal preparations are widely used, including herbal preparations containing flavonoids [32, 33].

Flavonoids are a group of polyphenolic compounds produced by plants as secondary metabolites. They often occur in glycosylated or esterified forms, have a basic 15-carbon skeleton consisting of C3 and C6 rings linked by a single bond, namely rings A and B connected by a third carbon ring [34].

A meta-analysis showed that flavonoids have a significant overall effect on depression (p = 0.004, Hedges' g = -0.487, 95% confidence interval from -0.814 to -0.160). Subgroup analysis showed that depressive symptoms significantly decreased when the flavonoid dose was 50–100 mg per day or the treatment duration was more than 8 weeks [35].

Various factors are involved in the pathogenesis of depressive disorder. The "monoamine hypothesis of depression" is a well-known theory explaining depressive disorder, which states that a decrease in the level of monoaminergic neurotransmitters in the brain, particularly serotonin and norepinephrine, is the primary cause of depression. However, current data indicate the involvement of several neural and hormonal pathways in the development of depressive disorder. Other factors include increased activation of the hypothalamic-pituitary-adrenal (HPA) axis, reduced regulation of brain-derived neurotrophic factor (BDNF), as well as dopaminergic and glutamatergic systems [36].

Many flavonols possess antidepressant and anxiolytic activity, possibly by increasing 5-HT and decreasing 5-hydroxyindoleacetic acid (5-HIAA) in the brain [37]. Quercetin inhibits hepatocarcinogenesis mediated by reactive oxygen species (ROS) by upregulating enzymatic (catalase, superoxide dismutase (SOD), glutathione peroxidase, paraoxonase) and non-enzymatic (total glutathione) antioxidant defense systems [38]. Astragalin significantly improved behavioral deficits in a chronic unpredictable mild stress (CUMS) model, promoted SIRT1 expression, and reduced levels of NF-κB p65, NLRP3, cleaved caspase-1, IL-1β, and gasdermin D proteins in the hippocampus [25]. It has been established that the biological effects of apigenin (a flavone) are related to gene transcription, protein expression, and enzyme activity levels, as well as a decrease in the loss of antioxidant enzymes in cells treated with streptozotocin [39]. Isoflavones, which are abundantly found in legumes, particularly formononetin and calycosin, are quite specific to plants of the *Astragalus* genus and can improve cognitive functions and alleviate depressive symptoms [40].

The obtained data indicates the need for more in-depth study of the mechanisms of antidepressant action of the biologically active compounds of the analyzed *Astragalus* species. The observed effect of the aqueous extract of *Astragalus varius* herb, combined with its diuretic action [24], may be recommended for the correction of chronic cardiovascular diseases.

Study Limitations

This study was conducted on male mice using a single-dose regimen for the analyzed aqueous and aqueous-alcoholic extracts, as well as the reference drug (amitriptyline). Therefore, further research on animals of both sexes is necessary, as well as an expanded range of doses to establish therapeutic efficacy.

CONCLUSION

The study determined the flavonoid content in aqueous and aqueous-alcoholic extracts from *Astragalus varius* herb (2.54 % and 9.31 %, respectively) and *Astragalus ovatus* herb (1.06 % and 10.34 %, respectively). An antidepressant effect of the aqueous-alcoholic extract of *Astragalus ovatus* was established with single and chronic administration at a dose of 100 mg/kg over 21 days. The aqueous-alcoholic extract of *Astragalus varius* demonstrated an antidepressant effect with chronic administration from days 8 to 21 of the experiment. The aqueous extract of *Astragalus varius* showed an antidepressant effect with chronic oral administration from days 15 to 21 of the experiment, while the aqueous extract of *Astragalus ovatus* showed no antidepressant activity. The antidepressant effect of the analyzed extracts from *Astragalus varius* and *Astragalus ovatus* herbs is likely due to the flavonoid content in them, which is confirmed by correlation relationships. Determining the mechanism of action of the BASs in the analyzed extracts requires further research.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTION

Ulyana A, Matvienko — conceptualization, invwstigation, data analysis of research, writing a draft manuscript, revision and editing of the text of the article; Alyona Yu. Karetnikova — definition of the concept, validation; Natalia A. Durnova — definition of the concept, writing a draft manuscript, revision and editing of the text of the article. All authors confirm that their authorship meets the international ICMJE criteria (all authors have made significant contributions to the development of the concept, research and preparation of the article, read and approved the final version before publication).

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