Anxiolytic and Antidepressant Effects of Aqueous Tincture of the Aerial Part of *Myosotis arvensis*

N. Yu. Polomeyeva, R. V. Gurto, O. S. Bryushinina, V. A. Slepichev, A. V. Kaigorodtsev*, V. Yu. Smirnov, N. I. Suslov, and V. V. Udut**

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 151, No. 5, pp. 540-543, May, 2011
Original article submitted February 3, 2010

Experiments on outbred albino mice showed a significant anxiolytic effect of aqueous tincture of the aerial part of *Myosotis arvensis* (*L.*) (*Boraginaceae*) in a single daily dose of 0.5 ml/kg. In a dose of 2 ml/kg, the tincture of *M. arvensis* aerial part exhibited an anxiolytic and antidepressant effect. In contrast to phenazepam, aqueous tincture of *M. arvensis* did not inhibit exploratory and motor activities.

Key Words: *Myosotis arvensis; anxiolytic effect; antidepressant effect; elevated plus maze; conflict situation*

Drugs with psychotropic activity are widely used in clinical practice. This group includes antidepressants, anxiolytics, psychostimulants, adaptogens, *etc.* Their active components are in the overwhelming majority of cases chemically synthesized substances. This fact determines numerous contraindications of many anxiolytics and antidepressants, sometimes causing drug resistance after long-term treatment limiting their use [9]. Drugs with similar effects, but of plant origin, are a lesser challenge for the organism; they are safer and do not induce dependence. In addition, according to statistical data, up to 80% population of the planet prefer drugs of plant origin [11,12].

Many drugs of plant origin are characterized by a mild sedative effect [2,4,5,10]. Antidepressants of plant origin widely used in the EC countries and the USA contain St. John wort (*Hypericum*) extract [6-8,11). According to traditional medicine, *Myosotis arvensis* is also characterized by a mild sedative effect and by a positive effect on mood, which dictates the need of more detailed study of its characteristics and psychotropic effect.

We studied the anxiolytic and antidepressant effects of *M. arvensis* aerial part aqueous tincture.

MATERIALS AND METHODS

Aqueous tincture of *M. arvensis* (MA) aerial part (1:20 raw material:extract proportion was obtained by extraction in water bath for 1 h at 70°C. Dry aerial part (sprout tops and some leaves) served as the raw material.

Experiments were carried out on 120 outbred male mice (20-25 g). The animals were divided into groups (10 per group) for analysis of the results of behavioral tests: control group, groups of animals treated with MA tincture in doses of 0.5 ml/kg ($MA_{0.5}$) and 2 ml/kg (MA_2), and groups treated with reference drugs. These doses were based on doses of plants with anxiolytic and antidepressant effects used in popular medicine.

The reference drugs were phenazepam (benzodiazepine tranquilizer; 1 mg/kg) and ginseng tincture (psychostimulant of plant origin; 1 ml/kg). The studies were carried out during the morning hours (from 9.00). MA tincture and the reference drug were administered

Institute of Pharmacology, Siberian Division of the Russian Academy of Medical Sciences; *Siberian State Medical University, Tomsk; **Tomsk Military Medical Institute, Russia. ***Address for correspondence:*** udutv@mail.ru. V. V. Udut

0007-4888/11/1515-0604

intragastrically for 4 days and on day 5 one hour before the experiment. Controls received water.

The anxiolytic effects of the extracts were evaluated in the elevated plus maze test and in Vogel conflict test. The maze had 4 perpendicular arms and a platform in the center. Two opposite arms were open and two others had walls on 3 sides (except the side facing the center) and a roof. The maze was fixed on a special stand at a height of 1 m above the floor. The mouse was placed on the central platform, after which its excursions to closed arms (CA) and open arms (OA) of the maze, summary time spent in CA and OA were recorded over 5 min. The anxiolytic effect of the tincture or drug was evaluated by the number of visits to OA and the time spent there.

Vogel conflict test implies conflict of two motivations: conditioned reflex (drinking) and instinctive reflex (avoidance of painful electrical shock). The drinking reflex was trained by leaving the animals without water for 2 days, after which they were put for 4 days into a plastic box for 20 min with a bowl of water in a niche in one of the walls. The mice received MA tincture and phenazepam from day 1 of training 1 h before training in the box. On the day of testing, the mice after MA dose were placed again into the box and a difference of potentials was created between the metal floor of the box and the electrode plunged in the bowl so that the animals felt a subthreshold electric shock of level when attempting to drink. The number of approaches and volume of water drunk were then recorded individually for each animal for 20 min. The anxiolytic activity was evaluated by the differences in the number of punished drinking episodes in the control and experimental groups.

The effects of the drug on "behavioral despair reaction" were evaluated in by response to unavoidable stress situation. The animals were placed in cylinders with water, and their stunning (immobilization) in a characteristic posture, called "behavioral despair reaction", was observed. The duration of immobilization was recorded over the first 6 min and during the subsequent 10 min. It is assumed that the antidepressant effects of the drugs can be predicted on the basis of shorter immobilization during the first 6 min, while changes in this parameter during the subsequent 10 min reflect psychostimulatory activity.

Changes in specific exploratory activity were evaluated in the open field test by comparing the number of movements in the control and experimental groups [1].

The results were processed by paired comparison by the Mann—Whitney test. The differences were considered significant at $p<0.05$.

RESULTS

M. arvensis tincture in a dose of 0.5 ml/kg prolonged the time spent by the animals in OA more than 2-fold in comparison with the control and with the reference drug and reduced the time spent in CA in comparison with the phenazepam group (Table 1). The number of ventures to the OA was maximum in $MA_{0.5}$ group. A trend to prolongation of the time spent in OA and number of entries to OA were recorded in MA_2 group. Hence, the data indicate a reduction of anxiety level in $MA_{0.5}$ group.

A significant increase in the number of punished water drinking episodes was observed in Vogel's conflict situation test in both experimental groups (Table 1). The number of punished water drinking episodes in $MA_{0.5}$ group increased 3.3 times in comparison with the control group. In MA_2 group, the number of punished drinking episodes increased 2.7 times compared to the control. By increase in the number of drinking episodes, both experimental groups exhibited the same level as animals receiving the reference drug (phenazepam).

These results indicate a clear-cut anxiolytic effect of MA tincture in doses of 0.5 and 2 ml/kg; it is noteworthy that the effect was more pronounced in $MA_{0.5}$ group.

In the behavioral despair test, all antidepressants stimulated motor activity during period 1 irrespective of the mechanism of action, thus reducing the duration of immobilization (Table 2). The duration of immobilization was 1.3 times shorter in $MA_{0.5}$ group in comparison with the control. In MA_2 group, the

TABLE 1. Behavioral Parameters of Mice in Elevated Plus Maze and in Vogel Conflict Tests ($M\pm m$)

Test	Parameter	Control	Phenazepam	$MA_{0.5}$	MA_2
Elevated plus maze	Number of ventures to OA	3.29±0.68	3.43±0.61	5.00±0.49	3.29±0.52
	Time spent in OA, sec	20.29±5.20	25.29±4.06	52.71±7.63*+	30.14±4.55
Vogel conflict test	Drinking episodes	6.14±0.67	18.0±1.5*	20.0±3.6*	16.57±2.52*
	Number of approaches to water bowl	10.57±1.21	15.86±0.86*	19.57±2.62*	18.71±1.69*

Note. $p<0.05$ compared to: *control, +phenazepam.

TABLE 2. Behavioral Parameters of Mice in Porsolt Behavioral Despair Test (*M*±*m*)

Group	6 min		10 min	
	number of immobilization episodes	duration of i immobilization, sec	number of immobilization episodes	duration of immobilization, sec
Control	5.00±0.52	230.60±16.86	7.50±0.97	453.30±27.08
$MA_{0.5}$	6.70±0.96	170.40±20.39*	8.30±0.75	397.40±37.19
MA_2	5.18±0.76	159.00±23.74*	7.73±1.01	388.55±41.98
Ginseng	4.70±0.72	157.80±26.51*	6.20±0.88	412.60±49.73

Note. *p<0.05 compared to the control.

TABLE 3. Behavioral Parameters of Mice in the Open Field Test, Sum of Minute 2 and Minute 3 (*M*±*m*)

Group	Motor activity		Exploratory activity	Mental status		Total motor activity
	horizontal	vertical	hole reflex	defecation	grooming	
Control	14.00±3.69	5.60±1.19	9.0±2.2	1.10±0.31	1.80±0.36	31.50±6.71
$MA_{0.5}$	24.6±1.9*+	5.90±0.85+	12.60±2.65+	1.70±0.33	0.80±0.25*	45.60±4.38+
MA_2	20.10±2.97+	4.80±1.35+	12.90±2.68+	0.80±0.29	1.00±0.54	39.60±5.74+
Phenazepam	9.50±4.71	1.00±0.56*	1.90±0.75*	1.40±0.34	0.80±0.36	14.60±6.13*

Note. p<0.05 compared to: *control, +phenazepam.

duration of immobilization during the first 6 min decreased by 1.4 times in comparison with the control group, remaining similar to that in animals receiving reference drug. A trend to reduction of immobilization time was observed in both experimental groups during the subsequent 10 min (Table 2).

Hence, MA tincture in a dose of 2 ml/kg exhibited a more pronounced antidepressant effect than 0.5 ml/kg with negligible psychostimulation in this experiment.

The open field test showed significant stimulation of the exploratory and total motor activity of animals receiving MA tincture in doses of 0.5 and 2 ml/kg and its inhibition in mice receiving phenazepam (Table 3). In addition, the total number of grooming acts (manifestation of displacement activity in animals and reflection of their mental strain) decreased in experimental groups. Reduction of the number of grooming acts can serve as an indirect indicator of anxiety reduction.

Hence, stimulation of exploratory and total motor activity in the open field test was observed in animals treated with MA tincture, while phenazepam reduced significantly this activity. In addition, a common trend to anxiety reduction was observed.

The study was carried out within the framework of Federal Target Program "Research and Scientific and Pedagogical Staff of Innovation Russia" for 2009-2013.

REFERENCES

1. Y. Bures, O. Buresova, and D. P. Houston, *Methods and Basic Experiments for Studies of Brain and Behavior* [in Russian], Moscow (1991).
2. T. A. Voronina and S. B. Seredenin, *Eksp. Klin. Farmakol.*, **65**, No. 5, 4-17 (2002).
3. V. A. Krauz, Yu. O. Fedotova, E. V. Zhokhova, and O. V. Ryzhova, *Rastit. Res.*, **43**, No. 3, 116-121 (2007).
4. V. A. Kurkin, A. V. Dubishchev, V. N. Yezhkov, and I. N. Titova, *Ibid.*, **43**, No. 3, 130-139 (2007).
5. M. D. Mashkovskii, *Drugs* [in Russian], Moscow (2005).
6. M. A. Morozova, A. G. Beniashvili, and N. B. Zharkova, *Psikhiatr. Psikhofarmakoter.*, **3**, No. 1, 32-34 (2001).
7. V. V. Rozhanets, *Narkologiya*, No. 7, 40-45 (2003).
8. A. Solovyova and E. Akarachkova, *Vrach*, No. 10, 66-68 (2007).
9. I. K. Solovyova, *Rus. Med. Zh.*, **14**, No. 5, 385-389 (2006).
10. S. N. Turishchev, *Rational Phytotherapy* [in Russian], Moscow (2000).
11. A. V. Ushkalova and T. S. Illarionova, *Farmateka*, No. 20, 10-14 (2007).
12. D. P. Briskin, *Plant Physiol.*, **124**, 507-514 (2000).