Study of the emotional state of the offspring of rats treated with aqueous plant extracts

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Abstract. The influence of biologically active compounds on the functional state of the central nervous system and the emotional component in rats can be evaluated by using behavioral methods, which are based on studying the exploratory behavior of animals in an environment new to them. One such method is the study of animal behavior in an elevated cross-shaped maze with open and closed arms and the Porsolt "despair" test. The aim of our study was to investigate the emotional state of the offspring of rats treated with aqueous plant extracts of Centella asiatica, Ginkgo biloba, Eleuterococcus and a mixture of aqueous extracts of Centella asiatica and Ginkgo biloba. The study of the emotional state of the offspring of rats treated with aqueous plant extracts was performed on 3 month old animals obtained in the experiment from males (20 animals) and females (50 animals), which received aqueous plant extracts at a dose of 30 mg/100 g of the animal body weight, in the volume of 1 ml for 30 days. Conclusions: Aqueous extracts of Centella asiatica, Ginkgo biloba and Eleutherococcus have pronounced nootropic activity, as the evaluation of animal anxiety level in the Elevated Cross Maze test and depression level in the Porsolt Despair Behavior test show a significant difference in the behavior of intact rats and offspring rats, receiving aqueous plant extracts as an additional load and the most pronounced effect is observed with the complex application of extracts of Centella asiatica and Ginkgo biloba.

1 Introduction

The influence of biologically active compounds on the functional state of the central nervous system and the emotional component in rats can be evaluated using behavioral methods, which are based on studying the exploratory behavior of animals in an environment new to them [1-5]. One such method is the study of animal behavior in an elevated cross-shaped maze (ECSM) that has open and closed arms and the Porsolt "despair" test [6-8].

The behavior of living organisms is built on the basis of satisfaction of leading needs and evaluation of achieved results. Individual behavior in animals includes a variety of

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behavioral acts that ensure the survival of the individual [9-11]. Forms of individual behavior include, for example, locomotion, shelter seeking, predator avoidance, grooming, and exploratory activity. The behavior of a particular animal is the result of the interaction of genetic factors and acquired experience [11-14]. It is believed that the elevated cruciform maze in rodents determines exploratory behavior, which reflects the desire to get acquainted with a new environment combined with caution and includes a cognitive component in the form of orientation in space [6, 15].

The Porsolt method of behavioral despair (helplessness) is a basic model for evaluating the effect of biologically active compounds - antidepressants [16]. The essence of the method is that animals, getting into water, begin to exhibit vigorous motor activity, aimed at finding a way out of an unpleasant situation. Then animals leave attempts to get out of the vessel and hang in water in a characteristic pose, remaining completely motionless or making insignificant movements, which are necessary to keep their muzzle above the water surface. This test makes it possible to determine the severity of the depressive state in an animal by the duration of the immobilization stage [16-18].

Various biologically active substances can modulate the animal's psychotropic activity, which can be manifested by sedation or psychostimulation (by decreased or increased motor and exploratory activity), tranquilizing or anxiolytic effects (by increased time in open ECSM arms and latent period before the animal starts exploring ECSM arms, and nootropic effects (ECSM arm patrol behavior indicators, dynamics of motor and exploratory activity).

In this connection, the *purpose* of our study was to investigate the emotional state of the offspring of rats treated with aqueous plant extracts.

The following *tasks* were to be solved for the purpose: to obtain offspring from rats which were daily injected with aqueous extracts of Centella asiatica, Ginkgo biloba, Eleuterococcus and a mixture of aqueous extracts of Centella asiatica and Ginkgo biloba as additional load during 30 days and to analyze the influence of these substances on the emotional state of the offspring.

3 Materials and methods of research

Experimental studies were conducted in accordance with the "Rules of work with the use of experimental animals" and in compliance with the principles of humanity set forth in the European Community directives (86/609/EC). The study of emotional state in the offspring of rats treated with aqueous plant extracts was performed on 3-month-old animals obtained in the experiment from males (20 animals) and females (50 animals), which received aqueous plant extracts in the dose of 30 mg/100 g of the animal body weight, in the volume of 1 ml for 30 days.

There were 5 groups of animals in the experiment and each group consisted of 10 females and 4 males: Group 1 - control, rats received daily distilled water in a 1 ml volume; Group 2 - animals received aqueous extract of Centella asiatica; Group 3 - aqueous extract of Ginkgo biloba; Group 4 - aqueous extract of Eleuterococcus; Group 5 - mixture of aqueous extracts of Centella asiatica and Ginkgo biloba. Aqueous extracts of Centella asiatica and Ginkgo biloba were purchased from «KorolevPharm» LLC. Aqueous extract of eleutherococcus was prepared at the Department of Pharmaceutical Technology from standardized raw materials. Standard methods were used to obtain dated females.

The study was conducted in an elevated cross-shaped maze, which allows us to assess the degree of anxiety resulting from stress caused by altitude and the unusualness of the situation - being placed in an open lighted space. The test was performed for 5 min and the following parameters were recorded: time spent in closed and open sleeves, number of

entries into open and closed sleeves, number of stands, hang-ups from the sleeve, defecations [19-22].

The level of depression was assessed in the Porsolt test of "despair," the essence of which is that after placing in a glass cylinder 20 cm in diameter and 40 cm high, 1/3 filled with water at 25±1 °C, the animal actively tries to get out of the water, swimming and making leaps up and dives under water in search of escape, then freezes in water in a characteristic posture (immobilization).

The severity of depression was assessed by the latent time to the first immobilization of the rat, the duration of immobilization, and the number of attempts to get out of the water assessed in the experiment for 5 minutes [19]. Statistical processing of the results was also performed.

3 Experimental results

The study of the degree of anxiety was carried out in an elevated cross-shaped maze, which allows you to assess the degree of anxiety resulting from the stress caused by height and the unusualness of the situation - being placed in an open lighted space. The results of the experiment are presented in Table 1.

Table 1. Anxiety scores of the offspring of rats treated with aqueous plant extracts in the Elevated Cross-Maze test.

Indicator	Control group	Group 1 (Centella asiatica)	Group 2 (gingko biloba)	Group 3 (eleutherococcus)	Group 4 (centella + gingko)
Number of swings	3,25±0,10	4,19±0,15[1]	3,82±0,13[1]	3,15±0,11	5,56±0,17[1]
Number of visits to open arms	1,67±0,05	2,45±0,08[1]	2,81±0,10[1]	1,79±0,06	3,74±0,13[1]
Number of visits to closed arms	1,25±0,04	1,68±0,06[1]	1,89±0,07[1]	1,19±0,04	2,15±0,06[1]
Dwell time in open sleeves, s	103,7±3,94	126,8±4,18[1]	133,5±4,81[1]	116,9±3,74[1]	139,2±5,01[1]
Dwell time in closed sleeves, s	95,6±3,35	76,5±2,45[1]	80,3±2,89[1]	99,7±3,49	61,3±2,32[1]
Time in the center, s	35,6±1,14	31,2±1,12[1]	32,5±1,24[1]	37,8±1,39	27,5±0,96[1]
Grooming in open sleeves, s	16,7±0,53	-	5,6±0,18[1]	11,9±0,43[1]	-
Grooming in closed sleeves, s	35,4±1,13	22,5±0,79[1]	26,7±0,93[1]	37,6±1,32	9,3±0,26[1]
Number of racks in open sleeves	-	-	-	-	-
Number of racks in closed sleeves	4,38±0,16	2,98±0,11[1]	3,12±0,12[1]	4,11±0,13	1,79±0,06

Note. In this table the differences are significant at P<0.05: 1 - in comparison with the control group animals

When evaluating the number of candles from the elevated cruciform labyrinth of rats, it was found that the offspring of rats receiving the extract of Centella asiatica showed 28.9% more (Manna-Whitney: U=90,500, Z = 2.856952 at p=0.000113) candles compared to control, the offspring of rats receiving the extract of ginkgo biloba - more by 17. 5% (Manna-Whitney: U=215,500, Z = 3.445741 at p=0.004115) of swellings, and the offspring of rats receiving eleutherococcus extract were characterized by approximately the same

number of swellings as intact rats. The offspring of rats receiving a mixture of plant extracts exhibited 71.1% more (Manna-Whitney: U=144,000, Z = 2.856584 at p=0.000000) hesitations compared to controls.

When estimating the number of visits to open arms by offspring of rats receiving aqueous plant extracts, it was found that the rats of the experimental group did so more frequently than the rats of the control group: the offspring of rats receiving Centella asiatica extract visited open arms more frequently than the intact rats by 46.7 % (Manna-Whitney: U=177,500, Z=4.455741 at p=0.002114), the offspring of rats receiving ginkgo biloba extract were 68.3 % more frequent (Manna-Whitney: U=86.00000, Z = 5.374139, at p=0.00000), the offspring of rats receiving eleutherococcus extract were 7.19 more frequent and the offspring of rats receiving a mixture of plant extracts were 123.9 % more frequent (Manna-Whitney: U=15.50000, Z = 6.415441, at p=0.00000).

Evaluation of the number of visits to closed arms by the offspring of rats receiving aqueous plant extracts revealed a similar trend to open arms: the offspring of rats receiving Centella asiatica extract visited closed arms more often than the control: the offspring of rats receiving Centella asiatica extract visited closed arms more often than intact rats by 34.4 % (Manna-Whitney: U=125,000, Z=2.844741 at p=0.002398), the offspring of rats receiving ginkgo biloba extract were 51.2% more frequent (Manna-Whitney: U=167,400, Z = 3.356696 at p=0.00000), offspring of rats receiving eleutherococcus extract were at the level of the control group, and offspring of rats receiving a mixture of plant extracts were 72.0% more frequent (Manna-Whitney: U=193,500, Z = 4.255152 at p=0.000015).

The offspring of rats receiving aqueous plant extracts spent more time in the open arms of the elevated cruciform labyrinth than the rats of the control group: the offspring of rats receiving the extract of Centella asiatica - more by 22.3 % (Manna-Whitney: U=99,000, Z = 2.988787 at p=0.000315) time, offspring of rats receiving ginkgo biloba extract increased by 28.7% (Manna-Whitney: U=132,000, Z = 4.444784 at p=0.003665), offspring of rats receiving eleutherococcus extract increased by 12.7% (Manna-Whitney: U=128.9000, Z = 4.588958 at p=0.003774), and the offspring of rats receiving a mixture of plant extracts were 34.2% greater (Manna-Whitney: U=197.000, Z = 5.488747 at p=0.000000) time.

The offspring of rats receiving aqueous plant extracts spent less time in the closed arms of the elevated cruciform labyrinth than the rats of the control group: the offspring of rats receiving the extract of Centella asiatica - less by 19.9 % (Manna-Whitney: U=96.0000, Z=2.987747, at p=0.000000) time, the offspring of rats receiving ginkgo biloba extract were 16.0% less (Manna-Whitney: U=157.80000, Z = 2.587741, at p=0.004668), the offspring of rats receiving eleutherococcus extract were approximately as intact rats, and the offspring of rats receiving a mixture of plant extracts were 35.9 % less (Manna-Whitney: U=166.6000, Z = 3.358858, at p=0.003447) time.

The offspring of rats receiving aqueous plant extracts, except eleutherococcus extract, spent less time in the center of the elevated cruciform labyrinth than the rats of the control group: the offspring of rats receiving Centella asiatica extract - less by 12.4 % (Manna-Whitney: U=124,5000, Z = 3.477541, at p=0.004698) time, the offspring of rats receiving ginkgo biloba extract were 9.5% less (Manna-Whitney: U=147.8000, Z = 3.699828, at p=0.000115), the offspring of rats receiving eleutherococcus extract were 6.2 % longer than intact rats, and the offspring of rats receiving a mixture of plant extracts were 29.5 % less (Manna-Whitney: U=156.3000, Z = 5.488726, at p=0.000128) time.

The offspring of rats receiving the aqueous plant extract of Centella asiatica and a mixture of aqueous extracts of Centella asiatica and Gingko biloba did not groom in the open arms of the elevated cruciform maze, and the offspring of rats receiving the aqueous extract of Ginkgo biloba and Eleuterococcus did groom, but its duration in time was less than that of controls by 66.5 % (Manna-Whitney: U=115,5000, Z = 3.654414, at

p=0.002111) and 28.7% (Manna-Whitney: U=177,5000, Z = 3.785874, at p=0.002114) respectively.

In the closed arms of the elevated cruciform maze, the rats of all groups performed grooming, but the offspring of the rats receiving aqueous plant extracts, except eleuterococcus extract, were characterized by less grooming time than the rats of the control group: the offspring of the rats receiving Centella asiatica extract - less by 36.4% (Manna-Whitney: U=156.2000, Z = 4.233531, at p=0.000111) time, the offspring of rats receiving ginkgo biloba extract less by 24.6% (Manna-Whitney: U=92.0000, Z = 5.632212, at p=0.000000), the offspring of rats receiving eleutherococcus extract were 6.4% greater than intact rats, and the offspring of rats receiving a mixture of plant extracts were 73.7% less (Manna-Whitney: U=105.3000, Z = 2.874147, at p=0.002954) time.

During the study, rats of all groups did not perform vertical stances in the open arms, but in the closed arms of the elevated cross-shaped labyrinth, the offspring of rats receiving aqueous plant extracts performed fewer vertical stances than intact rats: the offspring of rats receiving Centella asiatica extract performed 31.9 % less (Manna-Whitney: U=124.7000, Z = 3.477854, at p=0.000113), offspring of rats receiving ginkgo biloba extract less by 28.8% (Manna-Whitney: U=161.0000, Z = 2.458252, at p=0.000012), offspring of rats receiving eleutherococcus extract were 6.2% less than intact rats, and offspring of rats receiving a mixture of plant extracts were 59.1% less (Manna-Whitney: U=171.2000, Z =3.655852, at p=0.000145).

The level of depression was assessed in the Porsolt test of "despair," the essence of which is that after placing in a glass cylinder 20 cm in diameter and 40 cm high, 1/3 filled with water at 25±1 °C, the animal actively tries to get out of the water, swimming and making leaps up, dives under water in search of escape, then freezes in water in a characteristic posture (immobilization). The severity of depression is assessed by the latent time before the first immobilization of the rat, by the duration of immobilization, by the number of attempts to get out of the water, assessed in the experiment for 5 minutes (Table 2).

Table 2. Depression scores of the offspring of rats treated with aqueous plant extracts in the Porsolt Despair Behavior Test.

Indicator	Control group	Group 1 (Centella asiatica)	Group 2 (gingko biloba)	Group 3 (eleutherococcus)	Group 4 (centella + gingko)
Active swimming, s	162,43±5,68	185,34±6,12[1]	179,58±6,28[1]	165,97±5,31	203,91±6,53
Latency time, s	121,92±4,26	139,45±4,88[1]	135,21±4,73[1]	125,31±4,01	147,52±5,46[1]
Number of attempts to get out	18,34±0,66	24,38±0,86[1]	25,42±0,97[1]	19,25±0,62	29,45±0,91[1]
Passive swimming, s.	138,51±4,43	109,71±3,73[1]	113,94±3,87[1]	125,38±3,89[1]	85,74±3,00[1]
Time immobilization time, s	114,72±4,12	89,41±2,86[1]	82,65±3,06[1]	101,29±3,65[1]	51,92±1,87[1]
Boluses	4,93±0,14	3,09±0,09[1]	3,01±0,10[1]	4,00±0,13[1]	2,93±0,09[1]

Note. In this table the differences are significant at P<0.05: 1 - in comparison with the control group animals

When evaluating active swimming time, it was found that the offspring of rats receiving Centella asiatica extract showed 14.1% longer (Manna-Whitney: U=113.500, Z = 3.245514 at p=0.002567) swimming time compared to the control; the offspring of rats receiving ginkgo biloba extract showed 10.6% longer (Manna-Whitney: U=175.600, Z = 3.298584 at p=0.000000): U=175,600, Z=3.298584 at p=0.000000), and the offspring of rats receiving eleutherococcus extract were characterized by approximately the same swimming time as intact rats. The offspring of rats receiving a mixture of plant extracts exhibited 25.5% more (Manna-Whitney: U=151.3000, Z = 3.456565 at p=0.000011) swimming time compared to controls.

The offspring of rats receiving aqueous plant extracts, except eleutherococcus extract (latent time was as in the control), were characterized by longer latent time compared to the rats of the control group: the offspring of rats receiving Centella asiatica extract showed a 14.4% shorter latent time (Manna-Whitney: U=112,5000, Z = 3.245414, at p=0.000000); offspring of rats receiving ginkgo biloba extract showed 10.9% less (Manna-Whitney: U=135.6000, Z = 3.214241, at p=0.000113); progeny of rats receiving a mixture of plant extracts had 21.0% less (Manna-Whitney: U=141.6000, Z = 5.245575, at p=0.000000) latent time.

Evaluation of the number of escape attempts showed that the rats of the experimental groups made attempts more frequently than the control: the offspring of rats receiving Centella asiatica extract made attempts 32.9 % more frequently than the intact rats (Manna-Whitney: U=105,000, Z = 2.547746 at p=0.00000), the offspring of rats receiving ginkgo biloba extract attempted 38.6% more frequently (Manna-Whitney: U=193,200, Z=3.848957 at p=0.002114), offspring of rats receiving eleutherococcus extract were 4.9% more frequent, and offspring of rats receiving a mixture of plant extracts were 60.6% more frequent (Manna-Whitney: U=146,500, Z=4.847757 at p=0.00000).

The offspring of rats receiving aqueous plant extracts were characterized by shorter passive swimming time compared to the control group rats: the offspring of rats receiving Centella asiatica extract demonstrated 20.8% shorter time (Manna-Whitney: U=96.7000, Z = 2.966566, p=0.000009); the offspring of rats receiving Ginkgo biloba extract showed 17.7% shorter time (Manna-Whitney: U=109.7000, Z = 3.444544, p=0.00454): U=109.7000, Z = 3.444544, with p=0.003454); progeny of rats receiving eleutherococcus extract were 9.5% less (Manna-Whitney: U=118.6000, Z = 3.688584, at p=0.003201), and the offspring of rats receiving a mixture of plant extracts had 38.1% less (Manna-Whitney: U=129.4000, Z = 5.245541, at p=0.003222) passive swimming time.

The offspring of rats receiving aqueous plant extracts were characterized by shorter immobilization time compared to the control group rats: the offspring of rats receiving Centella asiatica extract demonstrated 22.1% shorter time (Manna-Whitney: U=134.8000, Z = 3.112141, at p=0.000000); the offspring of rats receiving Ginkgo biloba extract showed 27.9% shorter time (Manna-Whitney: U=117.9000, Z = 3.565858, p=0.002994): U=117.9000, Z = 3.565858, with p=0.002994); progeny of rats receiving eleutherococcus extract were 11.7% less (Manna-Whitney: U=148.0000, Z=3.856636, at p=0.000000), and the offspring of rats receiving a mixture of plant extracts had 54.7% less (Manna-Whitney: U=196.0000, Z=4.232252, at p=0.002222) passive swimming time.

Evaluation of the number of boluses during the test showed that the rats of the experimental groups were characterized by fewer boluses than the control: the offspring of rats receiving the extract of Centella asiatica - less by 37.3% (Manna-Whitney: U=99,000, Z = 2.998589 at p=0.002696), the offspring of rats receiving the extract of ginkgo biloba - less by 38.9% (Manna-Whitney: U=152,600, Z = 3.666654 at p=0.000000), offspring of rats receiving eleutherococcus extract less by 18.9 (Manna-Whitney: U=124,5000, Z = 3.775774 at p=0.004952), and offspring of rats receiving a mixture of plant extracts less by 40.6 % (Manna-Whitney: U=111,500, Z = 4.252535 at p=0.000003).

4 Discussion of results

The offspring of rats receiving aqueous plant extracts, except eleutherococcus extract, were more likely than the intact animals to hang down from the arms of the cross-shaped maze, to visit the open and closed arms of the maze more often than the rats of the intact group, and to spend more time in the open arms than in the closed ones, to spend less time in the center of the elevated cross-shaped maze, and not to groom in the open arms. In the closed arms of the elevated cruciform maze, rats of all groups groomed, but the offspring of rats

receiving aqueous plant extracts other than eleutherococcus extract were characterized by less grooming time than rats of the control group. In the course of the study, the rats of all groups did not perform vertical stances in the open arms, and in the closed arms of the elevated cross-shaped maze, the offspring of the rats receiving aqueous plant extracts performed fewer vertical stances than the intact rats.

In general, an increase in the time spent in the open arms of the setup and a decrease in the closed arms indicates a lower level of anxiety in the rats.

The level of depression was assessed in the Porsolt "despair" test. When assessing the time of active swimming, it was found that the offspring of rats receiving aqueous plant extracts, except eleutherococcus extract, demonstrated a longer swimming time compared to the control; were characterized by a longer latent time; a greater number of attempts to get out showed; a shorter passive swimming time, a shorter immobilization time compared to the rats of the control group. Also the rats of the experimental groups were characterized by a smaller number of boluses than the control. The above parameters were especially pronounced in the rats born from the females receiving the mixture of plant extracts.

5 Conclusions

Aqueous extracts of Centella asiatica, Ginkgo biloba and Eleutherococcus have a pronounced nootropic activity, as the assessment of animal anxiety level in the "Elevated cross maze" test and depression level in the "despair behavior" test by Porsolt show a significant difference in the behavior of intact rats and progeny rats received aqueous plant extracts as an additional load and the most pronounced effect is observed with the complex application of extracts of Centella asiatica and Ginkgo biloba.

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