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STUDYING THE EFFECT OF A COMBINATION OF MOTHERWORT DRY EXTRACT AND GAMMA-AMINOBUTYRIC ACID ON THE BEHAVIOR OF RATS

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Abstract

This work is devoted to the preclinical study of the psychotropic properties of a substance containing a combination of motherwort dry extract and gamma-aminobutyric acid (MDE+GABA).

It was found that MDE+GABA exhibits neurotropic activity in a wide range of doses under intragastric administration in the prophylactic mode for 14 days in rats. Administration of MDE+GABA at the dose of 2 mg/kg (in terms of GABA) produces a nootropic effect in acute stress modeled by the extrapolation escape test. When the dose of MDE+GABA is increased to 8 mg/kg nootropic effect is replaced by a sedative effect. The results of the open field test indicate that MDE+GABA at doses of 4 and 8 mg/kg exhibits a sedative effect, which at the dose of 4 mg/kg is combined with an anti-stress effect. The elevated plus maze test shown that the pharmacodynamics of MDE+GABA are characterized by the elements of anti-anxiety action, which are maximally manifested at the dose of 4 mg/kg. Thus, the pharmacological profile of the studied substance combines nootropic and deprivative effects.

Key words: *motherwort dry extract, gamma-aminobutyric acid, psychotropic properties, open field test, extrapolation escape test, elevated plus maze test.*

Introduction

Anxiety disorders (AD) are currently among the most common mental health problems in the world, occupying a leading position among all medical problems [3, 4, 21].

Anxiety is one of the most frequent phenomena accompanying clinical symptoms in patients with neuropsychic and psychosomatic disorders, as well as various psychological problems in healthy people. [28, 29].

Nowdays, the prevalence of mental disorders tends to further increase both in Ukraine and around the world [18, 19, 22]. Almost 25% of the world's population suffers from various mental illnesses, among which AD is one of the most common diseases. The latter are found in 8-15% of the population, which exceeds the indicators of affective pathology and dependence from psychoactive substances [21]. Studies have shown that AD can often provoke suicidal behavior in patients [9].

The average annual incidence of AD in the adult population is 5-15% according to the American Psychiatric Association, however, the true prevalence of this pathology is much higher. This is probably due to the fact that panic and generalized AD are accompanied by various somato-vegetative manifestations, they have a wide polymorphism of clinical symptoms that force patients to seek advice from doctors of different specialties: cardiologists, neuropathologists, gastroenterologists, endocrinologists, pulmonologists, gynecologists, as well as pediatricians [5, 22, 23, 24, 25, 30, 31].

Despite the possibility of using a wide range of pharmacotherapeutic agents for the treatment of AD (anxiolytics, antidepressants, sedatives, etc.), their use is often difficult due to a number of adverse reactions, namely, the possible development of addiction, drug dependence, withdrawal syndrome, insomnia, increased excitability, which can have a significant impact on compliance [24]. In this regard, it is relevant to search for means of «alternative therapy« of AD, which are considered herbal preparations [11, 32, 33, 34].

It is well known that phytopreparations have a number of advantages over synthetic ones, since they are characterized by a wide range of pharmacological properties, sufficient therapeutic efficacy, the ability to comprehensively affect the body and low toxicity [16, 17, 25, 26, 27].

Methods

The studies were carried out on the basis of the Central Scientific Research Laboratory of the NUPh (Ukraine, Kharkov).

The studied substance is a combination of motherwort dry extract and gamma-aminobutyric acid (MDE+GABA) on a pharmacologically inert carrier (sucrose). The substance was obtained and standardized at the Department of Pharmacognosy, NUPh, under the guidance of Doctor of Pharmaceutical Sciences, Professor O. N. Koshevoy.

The substance was standardized by the content of flavonoids (not less than 0.02% in terms of hyperoside), iridoids (not less than 0.06% in terms of harpagide acetate) and GABA using the method of high-performance thin layer chromatography (Table 1).

The study of the psychotropic properties of the combination MDE+GABA was carried out on 42 white nonlinear male rats of 220-250 g. Experimental animals were kept in standard cages, at a standard diet with free access to water and food, under a natural day-night light regime and an air temperature of 20-22°C [8].

All manipulations with animals were carried out in accordance with the requirements of the European Convention «On the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes» (Strasbourg. 1986, as amended in 1998), Law of Ukraine No. 3446 - IV of February 21, 2006, as amended «On the protection of animals from cruelty» and «Directive of the European Union 2010/63 EU on the protection of animals used for scientific purposes» [7, 13].

The combination MDE+GABA was administered to rats intragastrically using a special probe at doses of 2 mg/kg, 4 mg/kg and 8 mg/kg (in terms of GABA) in the form of an aqueous solution, at the rate of 1.0 ml per 100 g of animal weight.

As reference drugs, we used an official motherwort tincture (MT) produced by PJSC Pharmaceutical Factory «Viola», Ukraine and substance GABA (Sigma). MT was administered to experimental animals intragastrically at a dose of 0.3

ml/kg, based on its average daily therapeutic dose for humans, taking into account the coefficient of species sensitivity according to I.P. Ulanova [15], and substance GABA at a dose of 50 mg/kg [20].

The combination MDE+GABA and comparison drugs were administered to rats once a day for 14 days before the experiment.

The psychotropic properties of the MDE+GABA combination were studied using psychopharmacological tests «extrapolation escape», «open field» and «elevated cruciform maze».

To study the effect of the combination MDE+GABA and reference drugs on the cognitive functions of rats under stressful conditions, we used the extrapolation release test [1, 14]. The behavior of the rats was observed for 3 minutes, and the test results were assessed according to the following indicators: the latent period of diving of the animals under the lower edge of the inner cylinder (time of solving the problem) and the total number of rats that solved the problem. If the animal did not dive under the edge of the cylinder during the observation period, the latency period was considered equal to 180 s. The experiment involved 6 groups of animals (6 rats in each): intact control (IC); animals that were injected with the combination MDE+GABA at doses of 2 mg/kg, 4 mg/kg and 8 mg/kg; rats injected with MT and GABA. Due to the possibility of influencing the behavior of experimental animals of manipulations associated with the administration of the test substance and reference drugs, the rats of the IC group were injected with purified water at the rate of 1.0 ml per 100 g of animal weight.

The extrapolation release test has a high aversive potential and is accompanied by the triggering of an acute stress reaction in laboratory animals. Therefore, when conducting tests «open field» and «elevated cruciform maze», an additional group of intact animals was used to differentiate the effect of the studied agents on the behavior of rats at the background of acute stress and, separately, of the stressful situation itself. The animals that formed the IC group in the extrapolation escape test were used as the control pathology (CP) group in the open field and elevated cruciform maze tests.

When setting the «open field» test, each animal was placed in the center of the field and its behavior was observed for 3 min. During testing, the locomotor activity (the number of squares crossed), the tentative research activity – horizontal (the number of peeping into the holes) and vertical (the number of vertical stands), as well as the indicators of autonomic accompaniment of emotional reactions (the number of fecal boluses, urination acts, grooming episodes) were assessed [6, 12, 14, 15].

The ability of the combination MDE+GABA to influence manifestations of anxiety in rats was established using the «elevated cruciform maze» test. Before the test, the device (maze) was at a height of 1 m above the floor. Its central part and transparent compartments were illuminated with an electric lamp. After 5 min in a dark cage, a rat was placed in the center of the maze with its head to the open arm. The behavior of the animals was observed for 5 min. The study took into account: the latent period of entry into the dark chamber (s), the time spent in the dark arms (s), the time spent in the illuminated arms (s), the number of visits to the dark and illuminated arms, the vegetative accompaniment of emotional reactions (the number of fecal boluses, urination, grooming acts) [15].

Statistical processing of the research results was carried out using the STATISTICA 8,0 program. With a normal distribution, the significance of differences between the groups was assessed using the parametric Student's test (t) at the accepted level of statistical significance $p<0,05$ [2, 10].

Results and Discussion

The results of the «extrapolation disposal» test, which are shown in Table 2, reflect two main blocks of behavioral reactions in experimental animals: aversive reactions associated with fear motivation and the desire to avoid a stressful situation (latent period of implementation and the number of aversive movements) and the manifestation of a search strategy for solving a heuristic problem (purposeful diving under the edge of the installation cylinder).

As can be seen from Table 2, the latency period for the implementation of aversive movements in animals of the IC group was 4.33 s, and the average number of jumps inside the cylinder and attempts to dive was 22.66. At the same time, the latent time for

the task (diving under the edge of the cylinder) was 77.5 s. The escape criterion was reached by 33.33% of the rats in this group.

Under the influence of the combination MDE+GABA at a dose of 2 mg/kg, the most pronounced decrease in the latent period of diving in rats was by 33.6% ($p < 0.05$) relative to IC. There was also an increase in the number of animals in the group that reached the criterion of getting rid of up to 50%. The results obtained indicate the ability of the MDE+GABA combination to improve cognitive functions in rats, which is confirmed by an increase in the speed of solving a complex heuristic problem and the number of animals that coped with it.

At a dose of 4 mg/kg, the combination MDE+GABA increases the latent period of the onset of aversive movements by 3.23 times ($p < 0.05$) and decreases their average number by 2.96 times ($p < 0.05$) compared with IC. At the same time, the latter indicator is 2.5 times less than in animals that were injected with a combination of MDE+GABA at a dose of 2 mg/kg, and by 1.84, 1.76 and 1.60 times lower than in rats, who received a combination of MDE+GABA at a dose of 8 mg/kg, MT and GABA, respectively. This indicates that in animals of this group, the manifestations of motor activity caused by a sense of fear decrease. The release criterion was reached by 66.67% of rats who received a combination of MDE+GABA at a dose of 4 mg/kg, which is significantly higher than in the IC group ($p < 0.05$), while there was also a decrease in the latent time of diving under the edge of the cylinder by 26 , 5% ($p > 0.05$) compared with the control group.

The obtained results shows that a course of administration of a combination of MDE+GABA to rats at a dose of 4 mg/kg improves their cognitive functions and weakens the manifestations of the motor component of emotional reactions to an acute stressful situation.

At the highest of the studied doses (8 mg/kg), the combination MDE+GABA, like the reference drug MT, did not show a statistically significant effect on the main indicator of cognitive functions – the latency time for diving under the edge of the cylinder ($p > 0.05$ compared to IC for both groups). At the same time, positive changes were established at the part of the manifestations of aversive reactions: an increase in the latent period by 2.11 times ($p < 0.05$) and a decrease in their average number by 1.6 times ($p < 0.05$) compared with IC.

It can be assumed that the combination MDE+GABA is to a certain extent characterized by a sedative effect, which is demonstrated by preparations based on motherwort per se.

The results of studying the effect of the combination of MDE+GABA and reference drugs on the behavioral responses of animals in the «open field» test are shown in Table 3.

It was established that behavioral disturbances in rats of the CP group, caused by the stressful effect of the highly aversive test «extrapolation release», manifested themselves in a statistically significant decrease in locomotor and orientation-exploratory activity. The motor activity of the animals of the CP group decreased by 42.7% ($p < 0.05$) compared with the IC at the background of an acute stress reaction. The vertical component of search reactions (the number of standings) decreased by 71.7% ($p < 0.05$), and the number of holes examined by 62.6% ($p < 0.05$). At the same time, at the background of a general suppression of basic motor reactions, rats of the CP group demonstrated more pronounced compulsive behavior: the number of grooming acts statistically significantly increased by 4.22 times ($p < 0.05$) compared with IC. There was also a tendency towards an increase in the number of fecal boluses and urination. Together, these changes in the behavioral reactions of animals of the CP group reflect the typical features of maladaptive disorders that arise in rats in response to an acute stress reaction.

Under these conditions, the effect of the combination MDE+GABA in the studied doses and comparison drugs on the indicators of the "open field" test turned out to be different.

At the background of the administration of MT and GABA, the indices of locomotor and orientation-exploratory activity decreased even more than in the CP group. MT decreased the number of squares crossed by animals by 69.7% ($p < 0.05$) and GABA by 77.7% ($p < 0.05$) compared to IC. The suppression of the exploratory component of behavioral reactions was manifested by a decrease in the number of holes studied in MT rats by 56.6% ($p < 0.05$) and in GABA – by 71.7% ($p < 0.05$) compared with IC. The number of upright stands against the background of MT and GABA administration decreased by 3.39 and

by 7.34 times, respectively (p <0.05) compared with IC. At the same time, there were no statistically significant changes in the vegetative accompaniment of behavioral reactions of rats of both groups. Only animals with MT had an increase in the average number of grooming acts. According to this indicator, they were similar to the rats of the CP group.

This influence of MT and GABA can be regarded as a manifestation of their nonspecific sedative effect, which is characterized by inhibition of both the motor and emotional-cognitive components.

The combination of MDE+GABA also caused a decrease in locomotor activity in rats. At a dose of 8 mg/kg, there was a maximum decrease in the number of squares crossed by animals by 78.3% (p <0.05) compared with the IC group, and by 62.8% (p <0.05) with CP. When it was administered at a dose of 4 mg/kg, there was only a tendency towards a decrease in this indicator compared to the CP group, and at a dose of 2 mg/kg there were no changes in locomotor activity relative to CP.

In a similar way, the combination MDE+GABA also influenced the indicators of orientation-research activity: its administration to experimental animals at a dose of 8 mg/kg manifested itself in a significant decrease in the number of holes studied and a noticeable tendency towards a decrease in vertical postures relative to the CP group, which was not observed with the administration of lower doses.

Based on the obtained results, it can be concluded that the course of prophylactic administration of the combination of MDE+GABA at a dose of 8 mg/kg to experimental animals is accompanied by the manifestation of a sedative effect under conditions of acute stress, and the severity of this effect significantly decreases with decreasing dose.

Noteworthy is the decrease in grooming acts against the background of the administration of a combination of MDE+GABA at a dose of 4 mg/kg by 4.22 times (p <0.05) compared with CP. This indicates a decrease in the manifestations of compulsive behavior in animals, which is usually a characteristic sign of an acute stress reaction.

The «open field» test revealed the sedative effect of the MDE+GABA combination, which is maximally manifested at a dose of 8 mg/kg. At a dose of 4 mg/kg, the depressing effect of the combination of MDE+GABA is complemented by the anti-stress effect, which to a certain extent correlates with the results obtained in the extrapolation release test (Table 1).

The effect of the combination MDE+GABA and reference drugs on the behavior of rats in a state of acute stress was studied in the «elevated cruciform maze» test. The results are shown in the table 4.

It was shown that the latent period of entry of rats of the CP group into the darkened compartment of the device and the time spent in it were 9.83 and 284.5 s, respectively. Only 15.33 s of rats were in the lighted compartments, having made only 2.33 visits, which indicates a pronounced anxiety in animals of this group, and is manifested by early entry into the dark compartment and prolonged stay in the «safe» compartment. This behavior is a consequence of the «mink reflex» - an unconditioned reflex of rodents, which occurs in response to a stressful situation.

Both the reference drugs and the combination MDE+GABA at doses of 2 mg/kg and 8 mg/kg increased the latency period for entering the darkened chamber. At the background of their administration, the time spent by the animals in the dark compartments of the device also decreased. However, these changes were not statistically significant, which only indicates a tendency for them to exhibit an anti-anxiety effect. The combination MDE+GABA had a pronounced anti-anxiety effect at a dose of 4 mg/kg: the time spent by animals in the dark compartments of the device decreased by 1.3 times compared to CP (p<0,05).

There was no statistically significant effect of MDE+GABA and reference drugs on the autonomic accompaniment of emotional reactions.

Thus, the data obtained indicate that the course of prophylactic administration of the combination MDE+GABA at a dose of 4 mg/kg leads to a decrease in the general level of anxiety in rats against the background of acute stress.

Conclusions.

Under the conditions of an acute stress reaction, modeled using the extrapolation release test, it was found that the combination of MDE+GABA at a dose of 2 mg/kg improves the cognitive functions of

animals, and with an increase in the dose to 8 mg/kg, it exhibits only elements of a sedative effect.

The data obtained in the "open field" test indicate that the combination of MDE+GABA has a pronounced sedative effect, as well as an anti-stress effect (reduction of manifestations of compulsive behavior). Elements of anti-anxiety action, maximally manifested at a dose of 4 mg/kg.

Studies have shown that in the pharmacological profile of the new combination of MDE+GABA there is a combination of depriming and nootropic effects.

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Table 1. The content of the main biologically active substances in the studied substance

Biologically active substances	Content in 1 g
Flavonoids	0,00026 g
Iridoids	0,00067 g
GABA	0,00428 g

Table 2. Effect of combination MDE+GABA on rat behavior indicators in the extrapolation escape test (M ± m, n = 6)

Indicators	IC	MDE+GABA			MT, 0,3 ml/kg	GABA, 50 mg/kg
		2 mg/kg	4 mg/kg	8 mg/kg		
The latent period of the implementation of aversive movements, s	4,33 ± 0,33	3,83 ± 0,79 ***/°/°°	14,00 ± 2,32*/**	9,17 ± 1,87*	6,83 ± 1,62	11,17 ± 1,70*
Number of aversive movements	22,66 ± 3,12	19,17 ± 3,66°	7,67 ± 1,64*/°°	14,17 ± 1,74*	13,50 ± 2,23*	12,33 ± 1,65*
Diving latency, s	77,50 ± 1,50	51,00 ± 4,36*	55,50 ± 13,17	80,00 ± 0,00	68,60 ± 12,10	28,00 ± 0,00*
The number of rats in the group that solved the problem, %	33,33	50,00*	66,67*	16,67	83,33*	16,67

Notes. Statistically relevant differences (p <0,05): * – relevant to IC group data; ** – relevant to data from MT group; *** – relevant to data from the GABA group; ° – relevant to data from MDE+GABA group 4 mg/kg; °° – relevant to data from MDE+GABA group 8 mg/kg; n - the number of animals in the group

Table 3. Effect of combination of MDE+GABA on rat behavior indicators in the open field test (M ± m, n = 6)

Indicators (3 min)	IC	CP	MDE+GABA			НП, 0,3 ml/kg	GABA, 50 mg/kg
			2 mg/kg	4 mg/kg	8 mg/kg		
Locomotor activity:							
Squares	58,30 ± 6,34	34,00 ± 6,78*	33,00 ± 5,54 */°°	18,83 ± 6,92*	12,67± 4,81 */**	17,67 ± 4,00*	13,00 ± 5,96 */**
Indicative research activity:							
Holes	8,83 ± 1,14	2,50 ± 0,92*	4,00 ± 0,45 */°°	3,00 ± 1,46*	0,83± 0,65 */°°°	3,83± 1,25*	2,50 ± 1,52*
Standing	14,67 ± 1,15	5,50 ± 0,76*	6,17 ± 1,14 */#/°°	4,17 ± 1,80*	1,83± 0,65 */**	4,33± 1,50*	2,00 ± 1,43*
Emotional reactions and their vegetative accompaniment:							
Grooming	0,67 ± 0,21	2,83 ± 0,65*	0,83 ± 0,31	0,67 ± 0,21**	0,67 ± 0,33	2,34 ± 0,80*	1,00 ± 0,81
Fecal boluses	0,67 ± 0,49	1,50 ± 0,96	0,17 ± 0,17	1,17 ± 0,54	2,67 ± 1,20	1,17 ± 0,75	1,83 ± 0,91
Urination	0,17 ± 0,17	0,33 ± 0,21	0,33 ± 0,21	0,17 ± 0,17	1,17 ± 0,65	0,17 ± 0,14	0,67 ± 0,21

Notes. Statistically relevant differences (p <0,05): * – relevant to IC group data; ** – relevant to data from CP group; *** – relevant to data from the GABA group; ° – relevant to data from MDE+GABA group 4 mg/kg; °° – relevant to data from MDE+GABA group 8 mg/kg; °°° – relevant to data from MT group; # –relevant to data from the GABA group. n - the number of animals in the group.

Table 4. Effect of combination of MDE+GABA on rat behavior indicators in the test «elevated cruciform maze», (M ± m, n = 6)

Indicators (5 min)	CP	MDE+GABA			MT, 0,3 ml/kg	GABA, 50 mg/kg
		2 mg/kg	4 mg/kg	8 mg/kg		
Latent period of entry into the dark chamber, s	9,83± 2,89	40,33± 18,91	6,60± 2,86	90,00± 44,86	19,86± 9,92	35,00± 15,30
Time spent in illuminated sleeves, s	15,33± 3,12	33,17± 9,76	74,67± 25,22*	27,83± 14,05	70,00± 26,97	61,33± 38,24
Time spent in dark sleeves, s	284,50± 3,07	265,00± 9,89	222,67± 25,48*	264,67± 12,75	230,00± 26,97	164,67± 56,66
Visits to the dark sleeves	2,83± 0,21	5,00± 0,73 */**	3,50± 1,15	2,33± 0,76	4,33± 1,02	2,00± 0,86
Visits to the illuminated sleeves	2,33± 0,21	4,33± 0,61 */**	3,17± 1,45	2,00± 1,03	3,33± 0,95	1,50± 0,67

Notes. Statistically relevant differences (p <0,05): * – relevant to IC group data; ** – relevant to data from the GABA group; n - the number of animals in the group