Features of the functional activity of melissa officinalis (Melissa officinalis L.) and medicinal mixture based on it

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Abstract. Using a battery of behavioral models (a black-and-white chamber, an open field and an elevated plus maze), comparative studies of the degree of severity and orientation of the functional activity of melissa officinalis and medicinal mixture based on it were carried out. The work was performed on 30 white female laboratory rats of the Wistar line, weighing 190-220 g. In the case of melissa officinalis monoculture, a water-alcohol tincture of this drug was used and administered orally to the animal on the day of testing. For the preparation of the experimental herbal mixture, the herb of melissa officinalis was used as the basis, as additional components of the mixture - herbs of oregano (Origanum vulgare) and garden sage (Salvia officinalis), hawthorn berries (Crataegus) and common licorice root (Glycyrrhiza glabra) in the ratio 17:10:10:10:10 (per 100 g of experimental herbal mixture: 30 g of melissa officinalis and 17.5 g of the remaining components). The water extraction of the product was carried out in accordance with the existing GOST recommendations. The manifestation of a significant anxiolytic effect of melissa officinalis, manifested by 2 test systems, was revealed. It was found that in the anxiolytic effect of melissa officinalis, the antiphobic component is more pronounced compared to the manifestation of research activity. The experimental medicinal mixture based on melissa officinalis caused, along with an increase in the stress of rats in an unusual situation for them, a more pronounced manifestation of search and research and motor activity.

1 Introduction

Among the pharmacopoeia plants, the raw materials of which can be actively used as a basis for the creation of medicines is melissa officinalis. The pharmaceutical market in Russia is dominated by quite expensive medicines based on melissa, which are mainly of imported origin. In modern conditions, issues related to the use of own plant resources for the production of medicines are becoming particularly relevant. Melissa officinalis has been actively used and is used in both ancient and modern folk and official medicine [1].

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An analysis of the literature on the characteristics, properties and possibilities of using herbal raw materials based on medicinal melissa indicates both the diversity of the therapeutic effect of this plant and the need for a more detailed study of the mechanisms of action of drugs based on it and the possibility of application [2, 3, 4, 5]. A wide range of melissa's functional activity is determined by the diversity of its primary and secondary metabolites [6, 7]. It is known that both complex mixtures and preparations prepared on the basis of one plant raw material are used as medicinal herbal preparations. It is believed that treatment with multicomponent mixtures is more effective than monocultures [8]. At the same time, when using the mixture, it is necessary to take into account a whole range of factors, including the individual characteristics of the patient, the presence of concomitant diseases, allergies or intolerance to certain components of the medicinal mixture. In this case, the selection of phytopreparations becomes more problematic.

According to A.V. Alekseeva, combined medicinal products based on melissa officinalis, presented on the pharmaceutical market of the Russian Federation account for 88%; among foreign drugs, this figure is even higher – 96%, i.e., as the author of the work emphasizes, melissa officinalis is practically not considered as an independent plant, but only as a component. At the same time, there are experimental data that made it possible to develop monopreparations based on melissa officinalis in the form of tinctures, dry tablets, syrups, solution concentrates, etc. [9, 10]. Even more significant is the fact of the use of these drugs in pediatric practice [11]. The author emphasizes that the herb melissa officinalis is among the herbal preparations allowed for use for the treatment and phytotherapy of various childhood diseases. And at the heart of this preference are such qualities of melissa as a mild sedative effect, a wide range of therapeutic effects and the absence of side effects [12].

The task of comparative analysis of the degree of severity and the nature of the orientation of the neurotropic activity of melissa officinalis (monoculture) and herbal mixture based on it was set in the work.

2 Research methodology

The work was performed on 30 white female laboratory rats of the Wistar line, weighing 190-220 g. The animals were kept in standard vivarium conditions, in daylight and at a temperature of 22-24 °C.

The assessment of behavioral reactions of animals when exposed to medicinal extracts was carried out on 3 test systems widely used in preclinical studies of drugs: a black-and-white chamber (BWC), an elevated plus maze (EPM) and an open field (OF). The methods and possibilities of using these behavioral models and their high validity were described by us earlier when assessing the influence of the electrical components of the atmosphere (aeroions) on animals. [13, 14]. According to existing concepts, when using a battery of tests, measurements begin with the least stressful ones. Our previous work using a sequence of three test systems EPM, OF and BWC revealed that the BWC model has the least stress, and the greatest - an OF model, therefore, the following sequence of behavior models used was used in this study: BWC - EPM - OF. For the same purpose, only one model was tested per day.

When using a monoculture, the test substance was administered orally to the animal on the day of testing and testing began after 60 minutes. As a herbal preparation, water-alcohol solutions of melissa officinalis were used. The main difference between the method of preparing these water-alcohol infusions is the extraction of fresh vegetable raw materials (without drying), which allows to neutralize the change in its chemical composition and extract a large amount of BAS in its original form. The method of preparation of tinctures was described by us earlier [14]. To prevent the influence of ethyl alcohol contained in the

extracts under study on animal behavior, the extractant was distilled on a rotary evaporator (Heidolph, Germany) at 56 °C at 130 rpm.

Taking into account our own and literary data, a herbal mixture was compiled on the basis of melissa officinalis, which has pronounced neurotropic activity. Herbs of oregano (Origanum vulgare) and garden sage (Salvia officinalis), hawthorn berries (Crataegus) and common licorice root (Glycyrrhiza glabra) were used as additional components of the mixture. The basis for the choice of these components was the peculiarities of their functional activity. Thus, manifestations of anxiolytic effects are characteristic of oregano and garden sage, nootropic and neuroprotective effects of licorice are described, and hawthorn berries have proven themselves as a means of reducing emotional excitability and increasing blood circulation in the vessels of the brain and heart muscle.

To prepare 100 g of the experimental herbal mixture, 30 g of the herb melissa officinalis and 17.5 g of the remaining components were selected, thus, the ratio of components in the mixture was: 17:10:10:10:10. Water extraction from the compiled herbal mixture was carried out according to the recommendations of the state pharmacopoeia article "Infusions and decoctions". To do this, the mixture was filled with water in a ratio of 1:10 and infused for 15 minutes in a water bath. Next, the flask was removed from the bath and infused at room temperature for 45 minutes, then the extraction was filtered through a cotton-gauze layer. The infusion was stored for 2 days at a temperature of 13-15 °C.

The selection of herbal raw materials for the preparation of extracts was carried out in ecologically favorable areas of the Republic of North Ossetia-Alania in the period from June to July. The extracts were made the next day after the collection of plant raw materials.

In addition to the main drinking bowl with clean water, additional drinking bowls with 5-8% saccharin solution were installed for noninvasive administration of the test drug into each cell of the experimental animals. Animals were trained to sweetened water for 3 days. Then the animals of each group were placed in individual cages and, using a syringe without a needle, each rat was taught to drink from it. After the training period, at the required time, each animal was offered to drink the appropriate drug independently [15]. Behavioral reactions were recorded using digital video system No. VS1304.

As in all our animal studies, the ethical principles of animal experiments and the main provisions of the Helsinki Declaration are observed in the work. The research work was carried out in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, March 18, 1986), and the Order of the Ministry of Health of the Russian Federation No. 267 dated 19.06.2003. In the experimental part, the minimum allowable number of laboratory animals was used in accordance with the applied method of statistical processing of the results obtained.

Statistical data processing was carried out according to the Student's criterion for uncorrelated samples; the differences were considered significant at $p \leq 0.05$. All calculations were performed in MS Exsel 2016 and the SPSS software.

3 Research results

A comparative analysis of behavioral reactions of white laboratory rats was performed when using a monoculture of melissa officinalis and a medicinal mixture based on it, using as additional components herbs of oregano and garden sage, hawthorn berries and licorice root.

The control group of animals (n=10) received ordinary water, experimental animals, respectively, an infusion of melissa (experiment 1, n= 10) and an infusion of medicinal mixture (experiment 2, n= 10).

The results of statistical processing of data on the peculiarities of the manifestation of behavioral activity of animals in this setting of experiments are presented below.

Table 1. Features of the effect of herbal extracts on the behavioral activity of rats in the Black-and-white Chamber test system.

Behavioral characteristics	Monitoring		Experiment 1		Experiment 2	
	Statistical indicators					
	$M_1 \pm m$	p ($M_1 - M_2$)	$M_2 \pm m$	p ($M_1 - M_3$)	$M_3 \pm m$	p ($M_2 - M_3$)
Latent period of entering the dark part, s	14.05±4.5	>0.05	14.0±7.6	>0.05	7.23±1.3	>0.05
Number of peeks from the dark part, pcs	1.80±0.83	>0.05	4.25±0.73	**≤0.05**	5.67±1.26	>0.05
Time of peeks from the dark part, s	6.55±2.90	**≤0.05**	31.5±10.0	>0.05	11.27±5.79	**≤0.05**
Number of exits from the dark part, pcs	0.6±0.2	>0.05	1.25±0.50	**≤0.05**	1.33±0.27	>0.05
Time in the light part, s	21.45±4.88	**≤0.05**	66.65±17.10	**≤0.01**	43.1±3.2	>0.05
Time in the dark part, s	286.88±10.1	**≤0.01**	228.15±10.40	**≤0.05**	245.86±3.2	>0.05
Number of defecations, pcs	0.20±0.18	>0.05	0.25±0.21	>0.05	0.33±0.33	>0.05
Number of urinations, pcs	1.60±0.46	**≤0.05**	0±0	**≤0.05**	0±0	>0.05

Note: hereafter M_1 is the arithmetic mean for group I, M_2 is the arithmetic mean for group II, M_3 is the arithmetic mean for group III, m is the representativeness error. Experiment 1 – melissa officinalis extract, experiment 2 – herbal mixture.

According to the BWC test, there was a significant increase in the time spent in the light part of the chamber and, accordingly, a decrease in the time spent in the dark part both when using medicinal melissa and herbal mixtures (Table 1). In both cases, the number of urinations significantly decreased. The time of peeking out of the dark part of the chamber significantly increased only when using monoculture, and the number of peeking out and exits – when using herbal mixtures. Such data, in accordance with the existing interpretation of behavioral reactions in the BWC, are regarded as a decrease in the level of anxiety caused by the active factor (phytopreparations). A decrease in the number of urinations can also be considered as a vegetative reaction of the body to a decrease in the effect of a stressful factor caused by an experimental situation.

In a comparative analysis of the behavioral characteristics of animals in this test system, when using melissa and herbal mixture, no significant differences were detected in all the evaluated parameters, except for the time of peeking out of the dark chamber: in rats receiving herbal mixture (experiment 2), this indicator was 35% lower compared to the experimental group 1 (Table.1).

Table 2. Features of the effect of herbal extracts on the behavioral activity of rats in the Elevated Plus Maze test.

Behavioral characteristics	Monitoring		Experiment 1		Experiment 2	
	Statistical indicators					
	$M_1 \pm m$	p ($M_1 - M_2$)	$M_2 \pm m$	p ($M_1 - M_3$)	$M_3 \pm m$	p ($M_2 - M_3$)
Latent period of the first movement, with	4.20±0.59	**≤0.05**	11.2±2.3	**≤0.01**	0.46±0.09	**≤0.05**
Time in the center, s	9.7±1.3	**≤0.05**	3.7±1.9	**≤0.05**	5.24±4.6	>0.05
Number of visits to open sleeves, pcs	4.28±0.63	**≤0.05**	2.25±0.54	>0.05	6.3±1.4	**≤0.05**
Number of visits to closed sleeves, pcs	3.2±1.3	**≤0.05**	2±0.4	>0.05	8±4.61	**≤0.05**
Time of visits to open sleeves, s	66.06±10.70	**≤0.05**	23.4±10.1	**≤0.05**	144.5±11.5	**≤0.05**
Time of visits to closed sleeves, s	235.2±8.6	**≤0.01**	277.3±7.8	**≤0.05**	149.3±14.3	**≤0.05**
Number of dipping acts, pcs	2.80±0.34	**≤0.01**	0.50±0.43	**≤0.05**	8±2.07	**≤0.05**
Number of grooming acts, pcs	3.4±1.4	>0.05	2.25±0.25	>0.05	2.33±0.54	>0.05
Duration of grooming acts, s	32.2±15.5	>0.05	13.8±2.4	>0.05	16.06±1.56	>0.05
Number of the acts of freezing behavior, pcs	1.60±0.61	>0.05	0.50±0.25	>0.05	1.33±0.87	>0.05
Time of the acts of freezing behavior, s	23.4±9.4	**≤0.05**	4.25±2.20	>0.05	6.5±4.07	>0.05
Number of rears, pcs	1.8±0.4	>0.05	3.0±0.7	**≤0.05**	14.3±2.8	**≤0.05**
Number of defecations, pcs	1.0±0.9	>0.05	0±0	>0.05	1.0±1.24	**≤0.05**
Number of urinations, pcs	1.60±0.46	**≤0.05**	0.25±0.41	**≤0.05**	0.33±0.27	>0.05

According to the EPM test, unidirectional (decrease) significant changes ($p \leq 0.05$) were detected by such behavioral characteristics as time in the center and the number of urinations (Table 2). In other cases, the changes were either multidirectional or reliable only in one of the cases. Thus, the indicators reflecting the time of visiting open sleeves ($p \leq 0.05$) and the number of acts of dipping ($p \leq 0.01$) decreased when using melissa, and increased ($p \leq 0.05$) when taking the medicinal mixture; the latent period of the first movement ($p \leq 0.05$) and the time of visiting closed sleeves ($p \leq 0.01$) increased when using melissa and decreased in the case of using the mixture ($p \leq 0.01$, $p \leq 0.05$). Significant changes (decrease) in such indicators as the number of visits to open and closed sleeves and the time of freezing behavior were observed only when taking melissa officinalis ($p \leq 0.05$), and the number of rears (upright posture on hind legs) significantly increased when taking a complex mixture ($p \leq 0.05$).

Thus, according to the EPM test, the latent period of the first movement significantly increased in rats receiving melissa officinalis extract, the time spent in the center, the number of overhangs (dipping) and the duration of stay in the open sleeves of the maze decreased ($p < 0.05$). This kind of data is considered as a result of the oppression of search-oriented behavior due to the unusual situation and the stress of the behavioral model. At the same time, a decrease in the number and time of the acts of freezing behavior, as well as the number of urinations ($p < 0.05$) indicate a decrease in emotional excitability.

The changes observed when taking the medicinal mixture, in particular, a decrease in the latency period of the first movement, are considered in the literature as an increase in the speed of decision-making about the strategy of behavior [16]. An increase in time in the

center (open space) by almost 5 times, the time of visiting open sleeves and an increase in the number of rears and dipping is considered, on the one hand, as an increase in motor (locomotor) activity, and an increase in search and research activity. A decrease in the phobic component in the behavior of animals, an increase in stress resistance to an extreme situation for them in the form of elevation was manifested by a decrease in the number of urinations and a decrease in the time of visiting closed sleeves.

Table 3. Features of the effect of herbal extracts on the behavioral activity of female rats in the Open Field test.

Behavioral characteristics	**Groups of rats**					
	Group I		**Group II**		**Group III**	
	Statistical indicators					
	$M_1 \pm m$	**p ($M_1 - M_2$)**	**$M_2 \pm m$**	**p ($M_1 - M_3$)**	**$M_3 \pm m$**	**p ($M_2 - M_3$)**
Latent period of exit to the periphery, s	5.0±0.4	**≤0.01**	2.4±0.3	>0.05	3.8±2.45	>0.05
Number of crossed central squares, pcs	4.4±0.6	>0.05	6±2	**≤0.01**	16.33±0.71	**≤0.05**
Number of crossed peripheral squares, pcs	28±6.5	>0.05	40.3±4.7	>0.05	31±4.78	>0.05
Sum of crossed squares, pcs	32.4±4.2	>0.05	46.25±6.3	>0.05	38.66±1.43	>0.05
Vertical motor activity, pcs	2.2±0.71	>0.05	5.3±0.9	**≤0.05**	5.46±0.76	**≤0.01**
Number of grooming acts, pcs	3.4±0.93	**≤0.05**	0.75±0.20	>0.05	3±1.24	>0.05
Duration of grooming acts, s	27.9±52	**≤0.05**	9.35±2.5	**≤0.05**	9.33±5.13	>0.05
Number of the acts of freezing behavior, pcs	7.2±0.53	**≤0.001**	1.5±0.25	**≤0.05**	4±1.69	>0.05
Time of the acts of freezing behavior, s	59.5±4.31	**≤0.01**	9.25±2.8	>0.05	48±20.10	>0.05
Number of defecations, pcs	2.6±0.80	**≤0.01**	0.25±0.2	>0.05	2.33±0.97	**≤0.01**
Number of urinations, pcs	0.8±0.35	>0.05	0.5±0.4	>0.05	0.33±0.27	>0.05
Number of hole sniffing, pcs	1.8±0.72	**≤0.01**	4.5±0.25	**≤0.01**	10.66±1.65	**≤0.01**
Number of exits to the center, pcs	1.2±0.44	>0.05	2.25±0.8	>0.05	1.33±0.54	>0.05

According to the OF test, there was a significant decrease in the duration of grooming acts and the number of the acts of freezing behavior in relation to the control group of animals, and an increase in vertical motor activity and the amount of hole sniffing both when using medicinal melissa and herbal mixture (Table 3). A decrease in the number of grooming acts, the time of freezing, the number of defecations and the latent time of exit to the periphery was observed in animals that were watered with melissa infusion ($p \leq 0.01$), while the number of crossed central squares significantly increased when rats received infusion from herbal mixture ($p \leq 0.01$).

4 Result discussion

Summarizing the above data on the three test systems, we note the following features of the effect on the behavioral reactions of laboratory rats of medicinal melissa and herbal mixture

based on it. The behavioral models used in the work allowed us to identify 3 main groups of reactions manifested in changes in vegetative activity, search and research behavior and motor locomotor activity. To varying degrees of severity, these reactions were manifested when using both different test systems and tested herbal preparations.

The use of melissa tincture and herbal infusion determined a significant decrease in the phobic component in animal behavior due to the stressful situation created in all experimental installations used: open space, illumination, elevation. It is well known that these situations are traumatic for rodents due to the genetically determined features of their behavior [16]. Changes in emotional excitability, demonstrating a decrease in the fear reaction, were manifested both in vegetative (a decrease in the number of urinations and defecations) and in behavioral reactions associated with avoiding situations leading to stress: the duration of being in open and closed sleeves, in the light and dark parts of the installation, the time of visits to open and closed sleeves, changes in the ratio intersections of peripheral and central squares, manifestation of holding reactions (number of freezing). However, along with such behavioral activity, reactions were intensified when taking herbal preparations, demonstrating an increase in search and research (number of dipping, number of rears, sniffing holes, vertical motor activity) and locomotor (vertical motor activity, visiting open and closed sleeves) activities.

A comparative analysis of the behavior indicators of animals that had a significant character allowed us to identify the differences that occur when they receive a monoculture of medicinal melissa or herbal mixture (Table 1, 2, 3). According to the BWC, melissa officinalis caused a more pronounced effect of reducing fear in rats compared to the medicinal mixture, which manifested itself both in an increase in the time of peeking out of the dark compartment of the camera, and in the duration of being in the light (increase) and dark (decrease) compartments. A decrease in the number of urinations occurred with the use of both preparations (Table 1). The indicator reflecting the increase in motor activity was significantly increased only when using herbal mixture.

Changes in the EPM test were more pronounced when using the medicinal mixture. At the same time, in animals, along with a decrease in stress (a decrease in the number of urinations, the duration of visits to closed sleeves), there was an increase in search and research and locomotor activity: an increase in the number of rears, the number of dipping, the time of visits to open and closed sleeves, time in the center; judging by the dynamics of the latent period of the first movement (a significant decrease), the decision-making time increased (Table 2).

The totality of behavioral changes when using melissa infusion (reduction in the duration and number of grooming acts, number and time of freezing, reduction in the number of defecations) according to the OF test indicate, first of all, a decrease in overall emotional activity, a decrease in the reaction of fear to the experimental situation. Along with this, an increase in hole sniffing and vertical motor activity suggests an increase in research activity. The intake of herbal infusion also determined, along with a decrease in the phobic component in behavior (the duration of grooming and the number of acts of freezing), a simultaneous increase in research activity. However, a comparative analysis of the tinctures used reveals differences that manifest themselves, first of all, in a less pronounced decrease in the reaction of fear to the open space and more pronounced research activity (the number of surveys of holes increases by more than 2 times compared with the results of melissa) in animals, when taking an infusion from a herbal mixture.

This kind of data can be considered as a more pronounced antiphobic component of the anxiolytic effect of melissa officinalis monoculture compared to the medicinal mixture. When animals receive a medicinal mixture, along with a pronounced anti-stress manifestation of the drug, detected by all three test systems used, more significant changes in motor and especially search and research activity are observed in them.

Apparently, in order to enhance the pharmacological effect of the experimental medicinal mixture, it is necessary to take this feature into account and additionally use plant raw materials with a more pronounced effect on performance indicators.

5 Conclusions

1. The manifestation of a significant anxiolytic effect of melissa officinalis, manifested by 2 test systems, was revealed.
2. It was found that in the anxiolytic effect of melissa officinalis, the antiphobic component is more pronounced compared to the manifestation of research activity.
3. Experimental medicinal mixture based on melissa officinalis, along with an increase in the stress of rats in an unusual situation for them, a more pronounced manifestation of search and research and motor activity.

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