Nature and severity of the effect of herbal medicinal products based on Melissa officinalis L. on animal behaviour when administrating different drug dosages

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The purpose of this research is to study the impact of herbal medicinal products based on *Melissa officinalis* on the anxiolytic effect in animals at different dosages of the drug. The study was conducted on albino Wistar rats using a sequence of three behavioural test systems (black and white chamber, elevated cruciform maze and open field). The combined medicinal product included the following starting materials of herbal origin: *Melissa officinalis, Origanum vulgare, Salvia officinalis, Crataegus berries and Glycyrrhiza glabra root,* in the ratio 17:10:10:10:10. Two dosages of the drug were tested: 5 ml and 2.5 ml per 1 kg of animal weight. The active components of the starting materials of herbal origin were isolated through continuous extraction in the Soxhlet apparatus with ethyl alcohol; after that, the solvent was distilled in a rotary evaporator. The herbal preparation produced notable effects by alleviating stress caused by the experimental setup (reducing phobic reactions) and simultaneously enhancing locomotion as well as exploratory and searching activities. These effects were observed to varying degrees across all three behavioural models. A comparative analysis of the testing results at different dosages of the experimental herbal preparation demonstrated the comparability of quantitative changes in the recorded behavioural reactions. This data makes it possible to use a lower drug dosage (2.5 ml/kg) in subsequent studies. The developed complex herbal medicinal product can be used as a basis for creating phytopreparations with targeted anxiolytic effect. The experimental data obtained will subsequently help in choosing the optimal dosage for rational pharmacotherapy and prevention of psycho-emotional disorders in humans and animals.

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Keywords: behavioural activity, albino laboratory rats, herbal medicinal product, complex herbal preparation, extract, anxiolytic activity

INTRODUCTION

The significance of using herbal preparations is largely due to their broad range of effects, high safety profile combined with sufficient effectiveness, and their relative affordability and accessibility. Starting materials of herbal origin are used both as components of drugs and as an independent preparation. Such medicinal products demonstrate good efficacy in complex treatment and are used as a supportive therapy and for the prevention of diseases (Rajapakse & Davenport, 2019; Sarris, Panossian, Schweitzer, Stough & Scholey, 2011; Zhao, Zhang & He, 2017; Wang et al., 2021; Khwairakpam et al., 2018). They are better absorbed by the body without pronounced side effects, even after prolonged use (Nikolaev, Mondodoev & Shantanova, 2019; Sambukova, Ovchinnikov, Ganapolsky, Yatmanov & Shabanov, 2017; Ang, Song, Zhang, Lee & Lee, 2022; Kim & Lim, 2022; Laccourreye, Werner, Laccourreye & Bonfils, 2017).

Melissa officinalis, Origanum vulgare, Salvia officinalis, Crataegus sanguinea, Glycyrrhiza glabra – those are the plants that grow everywhere in Russia; they are affordable, easy to harvest and process. Since ancient times, the use of plants has been documented both as monocultures and as part of complex herbal mixtures. At the same time, it is possible both to alter the degree of impact of phytopreparations on the body and to change the nature and target of its action. In this context, it is crucial to examine how medicinal plants affect living organisms. This includes investigating not only the conditions and specifics of their direct effects and disease management but also identifying potential undesired effects and evaluating the duration of their impact. Earlier, we had achieved the varying degrees of anxiolytic effect of Melissa both when using it as a monoculture and as a basis in a complex herbal preparation. It was established that in the anxiolytic effect of *Melissa officinalis*, the reduction of phobic reactions is more pronounced compared to enhancing exploratory and searching activities (Khabaeva, Gappoeva & Chiviev, 2022). The experimental medicinal product based on *Melissa officinalis* caused a more pronounced manifestation of exploratory and searching activities and locomotion, along with an increase in the stress resistance of rats in an atypical setup (Gappoeva, Khabaeva, Gagloeva, Agayeva & Bugulova, 2023).

One of the methods of pharmacopoeial analysis of medicines is the quantitative measurement of the active substance and its constituent components. When administrating a medicinal product, including a herbal one, its dosage plays an important role, which, on the one hand, should induce a therapeutic effect, and on the other hand, should not cause adverse reactions and addiction.

MATERIALS AND METHODS

This study aims to evaluate the features of the functional manifestation of the herbal product based on *Melissa officinalis* on the behavioural characteristics of Wistar rats, taking into account the dosage of the drug used.

The research was performed on 30 albino Wistar male rats. All animals were kept in cages under standard vivarium conditions with free access to food and water.

As a tested herbal preparation, a combined extract was used, including herbal components from the following plants: *Melissa officinalis, Origanum vulgare, Salvia officinalis, Crataegus sanguinea, Glycyrrhiza glabra L.*

Extraction was carried out using multiple continuous extraction in a Soxhlet apparatus. The dry starting materials of *Melissa officinalis* (30%), *Salvia Officinalis* (17.5%), *Crataegus sanguinea* (17.5%), *Origanum vulgare* (17.5%), and *Glycyrrhiza glabra* (17.5%) were mixed in respective proportions. The resulting mixture in an amount of 30 g was placed in a paper cup in an extractor; extraction was carried out with ethyl alcohol 70% until the substance was completely isolated. The solvent was evaporated in a rotary evaporator (Heidolph, Germany) at 56oC by distilling the liquid at reduced pressure (Sarris et al, 2019).

Before the start of the experiment, animal body weight was measured using electronic scales to control their weight and to determine the dosage of the plant extract used. The weight of the animals ranged from 160 g to 200 g. The nature and severity of behavioural activity in albino laboratory rats receiving the tested herbal preparation were evaluated at different dosages of the drug (5 ml/kg and 2.5 ml/kg of animal weight) on three behavioural models.

The drug was administered by probing. For this purpose, a metal probe made of a syringe needle was used. A probe placed on a syringe with the drug was injected via the back wall of the pharynx along the oesophagus to the stomach. The permissible volume of liquid for this method is 1.5-2 ml. The drug was administered to two groups of rats: the first group (experiment 1) received the drug in an amount of 5 ml/kg, the second group (experiment 2) – 2.5 ml/kg; the third (control) group was not receiving

Table 1. Features of the effect of the herbal preparation on rat behaviour indicators in the black and white chamber test

Behavioural characteristics	Monitoring		Experiment 1 (5 ml/kg)		Experiment 2 (2.5 ml/kg)
	Statistical indicators				
	$M_1 \pm m_1$	P M_1-M_2	$M_2 \pm m_2$	P M_2-M_3	$M_3 \pm m_3$
Latent period, s	14.06±4.99	**p<0.001**	85.65±7.67	p>0.05	4.96±0.74
Number of peeks from the dark compartment, pcs	1.80±0.91	p>0.05	4.50±2.3	**p<0.05**	10.40±1.50
Duration of peeks from the dark compartment, s	6.55±3.22	p>0.05	3.42±2.03	p>0.05	3.22±4.75
Number of exits from the dark compartment, pcs	0.60±0.24	p>0.05	1.52±0.85	**p<0.05**	4.2±0.49
Time spent in the light compartment, s	0.20±0.20	p>0.05	0.25±0.25	**p<0.001**	0±0
Time spent in the dark compartment, s	2.00±0.32	**p<0.001**	0±0	p>0.05	0±0
Number of defecations, pcs	20.25±5.57	**p<0.001**	256.97±21.21	p>0.05	114.72±11.06
Number of urinations, pcs	286.88±T11.12	**p<0.001**	40.17±20.67	p>0.05	182.16±11.02

Note: Hereafter M_1 is the arithmetic mean for the control group, M_2 is the arithmetic mean for the group "Experiment 1", M_3 is the arithmetic mean for the group "Experiment 2"; m is a sampling error.

the drug. The behaviour of rats was evaluated on 3 test systems: black and white chamber (BWC), open field (OF) and elevated cruciform maze (ECM) according to the methods and interpretation of data described by us earlier (Khabaeva, Gappoeva & Chiviev, 2022; Gappoeva et al, 2023). The registration of behavioural reactions was carried out using the digital video system No. VS1304. Experiment was carried out in accordance with the rules of the European Convention for the Protection of Vertebrate Animals (Strasbourg, March 18, 1986) and the Order No. 267 of the Ministry of Healthcare of the Russian Federation dated 19.06.2003. The minimum allowable number of laboratory animals was used in accordance with the utilized methods of statistical data processing. Statistical analysis of the data was carried out using the SPSS and MS Excel 2016 software and according to the Student's t-criterion. The differences were considered significant at $p<0.05$.

RESULTS

The BWC test system allows us to evaluate the physiological effects of the drug under study, which are manifested in the natural desire of animals to avoid open and brightly lit spaces. It is logical that the rats of the control group spent only about 7% of the testing time in the light compartment, half of which was the latent period of entering the dark part, and the time and number of peeks from there were minimal, which characterizes the normal behaviour of animals in uncomfortable conditions of the chamber (Table 1).

In rats receiving 5 ml/kg of the extract of the combined herbal preparation, there was a significant increase in time spent in the light compartment and a decrease in the time spent in the dark compartment. In the control group, this indicator was 20.25 seconds; in the experimental group, it was 256.67 seconds, i.e. the duration of stay in the light part of the chamber increased by more than 12 times. At the same time, the period spent by the animals in the dark compartment got shorter (by 7 times). The latent period also significantly increased – from 14.06 to 85.65, and the number of urinations dropped to 0. The results of assessing the behavioural reactions of animals when administering the herbal product at the dosage of 2.5 ml/kg (experiment 2) demonstrated more significant changes in several indicators (the number of peeks and exits from the dark part of the

Table 2. Features of the effect of the herbal preparation on rat behaviour indicators in the elevated cruciform maze test

Behavioural characteristics	Monitoring		Experiment 1 (5 ml/kg)		Experiment 2 (2.5 ml/kg)
	Statistical indicators				
	$M_1 \pm m_1$	P M_1-M_2	$M_2 \pm m_2$	P M_2-M_3	$M_3 \pm m_3$
Latent period of the first movement, s	4.22±0.65	**p<0.01**	0.55±0.08	**p<0.001**	3.24±0.51
Number of visits to open maze arms, pcs	2.80±0.37	**p<0.05**	6.25±1.31	p>0.05	4.00±0.32
Number of visits to closed maze arms, pcs	4.00±0.32	p>0.05	5.50±0.96	p>0.05	4.60±0.60
Duration of visits to open maze arms, s	54.00±16.71	p>0.05	98.70±10.49	p>0.05	97.08±22.41
Duration of visits to closed maze arms, s	241.13±6.4	**p<0.01**	177.12±13.33	p>0.05	213.35±23.04
Number of dipping acts, pcs	2.80±0.37	**p<0.001**	17.00±2.27	p>0.05	14.20±1.24
Number of grooming acts, pcs	3.40±1.54	p>0.05	2.50±1.19	p>0.05	1.80±0.37
Duration of grooming acts, s	29.58±16.52	p>0.05	19.62±3.81	p>0.05	20.46±3.27
Number of the acts of freezing behaviour, pcs	1.60±0.68	**p<0.01**	6.0±0.91	p>0.05	10.00±1.41
Duration of the acts of freezing behaviour, s	23.39±10.32	p>0.05	14,751.13	p>0.05	22.86±2.74
Number of rears, pcs	1.80±0.49	**p<0.001**	17.25±0.85	p>0.05	14.80±1.28
Number of defecations, pcs	1.00±1.00	p>0.05	1.00±1.00	p>0.05	1.40±1.72
Number of urinations, pcs	1.6±0.5	p>0.05	0.25±0.25	p>0.05	0.20±0.20
Time spent in the centre, s	8.20±1.95	p<0.01	18.02±3.78	p>0.05	18.46±2.47

Source: compiled by the authors

chamber, the number of defecations). However, the time spent in the light compartment was significantly shorter, and the time in the dark compartment was significantly longer compared to data from the experimental group 1 (5 ml/kg).

In general, the administration of the drug at both dosages caused an increase in the duration of stay of animals in the light compartment, and at the same time, a decrease in the duration of stay in the dark compartment. The number of peeks and exits from the dark compartment increased. It is known that animals with a highly mobile nervous system are characterized by avoidance of open spaces and a hiding strategy induced by fear, therefore, the observed changes in behavioural reactions in response to the herbal product administration should be interpreted as a decrease in the manifestation of the phobic component in their behaviour.

The ECM test, like the BWC, is based on the animal's preference for either dark or light spaces (open and closed maze arms of the test system). In addition to bright and open spaces, the stress factor of this behavioural model is the elevation of the maze above the floor, which causes rats to have a natural fear of heights and determines their increased anxiety in these conditions. Furthermore, the test allows you to evaluate the locomotion and exploratory activity of the animals in a situation unusual for them (Table 2).

Table 3. Features of the effect of the herbal preparation on rat behaviour indicators in the open field test

Behavioural characteristics	Monitoring		Experiment 1 (5 ml/kg)		Experiment 2 (2.5 ml/kg)
	Statistical indicators				
	$M_1 \pm m_1$	P M_1-M_2	$M_2 \pm m_2$	P M_2-M_3	$M_3 \pm m_3$
Latent period of exits to the periphery, s	6.02±1.08	**p<0.05**	1.35±0.09	**p<0.05**	2.34±0.25
Sum of crossed squares, pcs	32.4±7.16	**p<0.05**	64.0±5.4	p>0.05	77.40±4.67
Vertical motor activity, pcs	2.20±0.97	**p<0.001**	19.5±2.22	p>0.05	21.20±1.65
Number of grooming acts, pcs	3.4±0.98	p>0.05	2.25±0.75	p>0.05	2.00±0.70
Duration of grooming acts, s	27.96±5.99	p>0.05	23.95±5.6	p>0.05	17.18±4.72
Number of the acts of freezing behaviour, pcs	7.20±0.58	**p<0.01**	3.00±1.08	p>0.05	3.40±1.20
Duration of the acts of freezing behaviour, s	61.75±6.47	**p<0.01**	20.9±7.16	p>0.05	34.28±13.25
Number of defecations, pcs	1.80±1.20	p>0.05	0±0	p>0.05	0±0
Number of urinations, pcs	0.80±0.37	p>0.05	0±0	p>0.05	0±0
Number of well inspections, pcs	1.80±0.80	**p<0.01**	14.5±1.32	p>0.05	14.00±1.22
Number of crossed central squares, pcs	4.4±0.75	**p<0.01**	14.50±1.32	p>0.05	14.20±1.39
Number of crossed peripheral squares, pcs	28.00±7.19	p>0.05	49.50±6.54	p>0.05	63.20±4.26
Number of exits to the centre, pcs	1.20±0.49	**p<0.01**	3.75±0.48	p<0.05	5.60±0.68

Source: compiled by the authors

With 5 ml per kg of animal weight in the ECM test, the latent period of the first movement decreased by 7.5 times in rats receiving the herbal preparation (Table 2). The number of visits to the open arms of the test maze significantly increased ($p<0.05$) and the time of visits to closed maze arms significantly decreased ($p<0.01$). This data is considered as a decrease in the animals' genetically determined level of anxiety resulting from a stressful situation.

Along with these indicators, the number of dippings and rears increased ($p<0.001$); at the same time, the duration of stay at the centre increased (Table 2). In accordance with the existing test interpretation of behavioural reactions, such dynamics in behaviour indicates the activation of locomotion as well as exploratory and searching activities of animals following the decrease in fear reactions associated with open space and heights. The results of a quantitative assessment of the behaviour of rats in the ECM after administration of the tested herbal product at the dosage of 2.5 ml/kg (experiment 2) showed similar dynamics in almost all recorded behavioural reactions and the absence of significant differences between the two experimental groups.

The use of the OF test is justified by the possibility of a precise assessment of the motor activity of animals, which is reflected in the number of rears, crossed squares and exploration (the number of inspections of wells at the bottom of the arena). The stress of this test is in the unusual setup and a large open brightly lit space, which is several times larger than the space of the cage where the animals are usually kept. Given that the OF test is the most stressful behaviour model for rodents, this testing was performed last.

A comparative analysis of the test results in the model OF system in two experimental groups also did not reveal significant differences in the majority of animal behavioural reactions when administered the herbal product at different dosages (Table 3). The exception was the data on the latent period of exit to the periphery ($p<0.05$), and the number of exits to the centre ($p<0.05$); the significant difference (increase) in the number of crossed peripheral squares compared with the control group was revealed only at the dosage of 2.5 ml/kg ($p<0.05$). The animals from the control group showed average locomotor activity and essentially did not visit the central compartments, staying in the peripheral part. There was practically no exploratory activity, and a strategy of concealment was largely observed (Table 3).

In general, in the experimental groups, under the influence of the herbal preparation, regardless of its dosage, significant changes in animal behaviour occurred and they were quantified in the recorded parameters. The tested herbal preparation caused an increase in the motor activity of animals: a significant increase in horizontal motor activity, in the number of crossed central squares and exits to the centre. The duration and number of freezing behaviour acts were also significantly reduced. The increase in exploratory activity was reflected in an increase in the number of inspections of the wells at the bottom of the arena and an increase in vertical motor activity (rears). As for the grooming indicators and the latent period of exits to the periphery, all changes turned out to be insignificant. Therefore, the herbal product caused a significant increase in locomotion, exploratory and searching activities and a decrease in manifestations of fear reactions (hiding behaviour).

DISCUSSION

It is known that the most pronounced manifestations of the psychoemotional state of animals in response to stress are species-specific innate reactions in the form of changes in their behaviour and vegetatics. By employing various categories of behavioural tests, we identified a set of behavioural and vegetative responses that characterize the stress state in albino laboratory rats when exposed to unfamiliar situations. These manifestations include reduced motor activity, a preference for dark compartments, frequent freezing, and increased defecation and urination.

The use of a herbal preparation based on *Melissa officinalis* caused an anxiolytic effect in animals, manifested as an increase in locomotion, the formation of exploratory and searching behaviour and a decrease in fear reactions. In all behavioural models, after administration of the herbal product, the varying degrees of changes in the functional status were recorded, indicating stabilization of the emotional state of animals put into an atypical situation. A decrease of fear was manifested in vegetative reactions (a decrease in the number of defecations and urinations) and in the behaviour of rats (being in open and closed maze arms, in light and dark compartments of the set-up, changes in the ratio of the crossing of peripheral and central squares, etc.). Moreover, the behaviour associated with the exploratory, searching and motor (locomotor) activity of the tested rats significantly changed. These reactions were also manifested in all three behavioural models (the number of acts of dipping, number of rears, sniffing of wells, vertical motor activity, visiting open and closed maze arms, duration and number of peeks from dark compartments).

When using medicinal plants as a basis for creating combined preparations, the determining factors, along with the qualitative characteristics of the product, are its dosage and the quantitative ratio of its various herbal components. Information regarding the dosage of phytopreparations, especially plant-based products, is ambiguous and varies among researchers; the dosage may depend on the type of plant, estimated parameters, and the specific biological effects (Halien, Anderson, Kim & Blanc, 2002; Niggemann & Grüber, 2003). When determining the dosage of the drug, we proceeded from previously performed experimental studies using herbal tisanes and infusions, in the form of monoculture or herbal preparation, and minimizing the impact on the animal's body (Khabaeva, Gappoeva & Chiviev, 2022; Gappoeva et al, 2023).

The comparative analysis of the behavioural reactions of rats when administering different dosages of the herbal product (5 ml/kg and 2.5 ml/kg), with rare exceptions, showed insignificant differences in all three test systems used in the work (Tables 1, 2, 3). When using a lower dosage of the herbal preparation, there remains a significantly pronounced tendency for stabilisation of the emotional and vegetative state of the animals, manifested in a decrease in fear reactions, increased motor activity and an active genetically determined orientative-trying reaction in various forms of behaviour in the test systems used. Furthermore, in some cases, when quantifying behavioural reactions, a more pronounced effect of the herbal preparation was observed in the case

of its lower dosage: the number of exits and the duration of peeking out of the dark compartment (BWC test), the number of crossed squares and the number of exits to the centre (OF test). The data available in the literature and practical therapy on possible undesirable effects of medicinal plants can be determined by the functional properties of the plant itself, the occurrence of a negative cumulative effect, the form of the drug used, and its dosage. In this regard, it seems appropriate to use the herbal preparation in a lower dosage where its therapeutic effectiveness remains unchanged.

This data makes it possible to use a lower drug dosage in subsequent studies in animals. By doing so, two effects are achieved: the degree of exposure to an external factor (drug) on the body decreases and the cost of vegetable raw materials lowers.

CONCLUSION

A combined herbal preparation with *Melissa officinalis* as the main active ingredient led to a reduction in the fear-related symptoms exhibited in the vegetative reactions of animals, and also increased their locomotion and exploratory and searching activity in all three stress-inducing behavioural models. A comparative evaluation of the functional activity of the tested herbal preparation showed no significant differences in the dynamics and quantitative parameters of physiological responses in albino laboratory rats across various behavioural test systems, regardless of the dosage of the experimental herbal preparation used (5 ml/kg and 2.5 ml/kg).

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A Melissa officinalis L. gyógynövényalapú készítmények állati viselkedésre gyakorolt hatásának jellemzői és súlyossága különböző dózisok esetében

A kutatás célja a Melissa officinalison alapuló növényi gyógyszerek állatokban kifejtett szorongásoldó hatásának vizsgálata volt különböző dózisokban. A vizsgálatot albínó Wistar patkányokon végeztük három viselkedési tesztrendszer (fekete-fehér kamra, emelt keresztpalló, porond) segítségével. A kombinált gyógyszerkészítmény a következő növényi eredetű kiindulási anyagokat tartalmazta: Melissa officinalis, Origanum vulgare, Salvia officinalis, Crataegus bogyók és Glycyrrhiza glabra gyökér, 17:10:10:10:10:10:10 arányban. A hatóanyag két adagját vizsgáltuk: 5 ml és 2,5 ml 1 kg állattömegre vetítve. A növényi eredetű kiindulási anyagok aktív komponenseit Soxhlet készülékben, etil-alkohollal történő folyamatos extrakcióval izoláltuk; ezt követően az oldószert rotációs párologtatóban desztilláltuk. A gyógynövénykészítmény enyhítette a kísérleti elrendezés okozta stresszt (csökkentette a fóbiás reakciókat), és egyidejűleg fokozta a helyváltoztatást, valamint a felfedező és kereső tevékenységet. Ezek a hatások különböző mértékben voltak megfigyelhetők mindhárom viselkedési modellben. A vizsgálati eredmények összehasonlító elemzése a kísérleti gyógynövénykészítmény különböző dózisainál hasonlóságáz. Ezek az adatok lehetővé teszik, hogy a későbbi vizsgálatokban alacsonyabb dózist (2,5 ml/kg) alkalmazzunk. A kifejlesztett komplex növényi gyógyszerkészítmény alapul szolgálhat célzott anxiolitikus hatású fitopreparátumok létrehozásához. A kapott kísérleti adatok a későbbiekben segítenek az optimális dózis kiválasztásában a racionális farmakoterápia és a pszicho-emocionális zavarok megelőzése érdekében emberekben és állatokban.

Kulcsszavak: viselkedési aktivitás, albínó laboratóriumi patkányok, növényi gyógyszer, komplex növényi készítmény, kivonat, anxiolitikus aktivitás