INFLUÊNCIA DA *PATRINIA SCABIOSIFOLIA* NA RESISTÊNCIA DE CAMUNDONGOS À VIBRAÇÃO DE CORPO INTEIRO

INFLUENCE OF *PATRINIA SCABIOSIFOLIA* ON THE RESISTANCE OF MICE TO WHOLE-BODY VIBRATION

ВЛИЯНИЕ ПАТРИНИИ СКАБИОЗОЛИСТНОЙ НА РЕЗИСТЕНТНОСТЬ МЫШЕЙ К ДЕЙСТВИЮ ОБЩЕЙ ВИБРАЦИИ

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RESUMO

O objetivo do estudo foi avaliar a eficácia de *P. scabiosifolia* na correção do estado fisiológico e psicossomático de camundongos expostos a vibrações agudas e prolongadas em todo o corpo. O efeito de uma tintura de 40% água-álcool a partir das raízes de *P. scabiosifolia* foi investigada em camundongos CD-1 sob a influência da vibração de corpo inteiro induzida por um agitador ou exposição ao transporte rodoviário. Componentes emocionais, orientados à exploração e, motor comportamentais foram observados em campo aberto. O desempenho físico foi avaliado pela duração da natação até a fadiga com um peso de 7% conectado à raiz da cauda. Os índices metabólicos foram corticosterona sérica, glicogênio, lactato e trifosfato de adenosina do fígado e do músculo esquelético. A droga administrada por via oral na dose de 5 ml / kg otimizou as reações comportamentais dos animais, aumentou seu desempenho no exercício, melhorou o estado hormonal e o metabolismo energético no fígado e músculo esquelético e tecidos sob a influência da vibração do corpo inteiro. A tintura de *P. scabiosifolia* possui um efeito vibroprotetor e pode ser recomendada para a prevenção e tratamento de distúrbios neurofisiológicos causados por vibração de corpo inteiro, em combinação com terapia básica.

Palavras-chave: *Patriniascabiosifolia* Fisch. exLink, reações comportamentais, desempenho no exercício, metabolismo, vibração em todo o corpo

ABSTRACT

The aim of the study was to evaluate the effectiveness of *P. scabiosifolia* in correcting the physiological and psychosomatic state of mice exposed to acute and prolonged whole-body vibration. The effect of a 40% water-alcohol tincture from the roots of *P. scabiosifolia* was investigated in CD-1 mice under the influence of whole-body vibration induced by a shaker or exposure to road transportation. Emotional, exploratory-oriented and motor behavioral components were observed in the open field. Physical performance was evaluated by the duration of swimming until fatigue with a 7% weight attached to the tail root. Metabolic indices were serum corticosterone, glycogen, lactate and adenosine triphosphate of the liver and skeletal muscle. The orally administered drug at a dose of 5 ml/kg optimized the behavioral reactions of the animals, increased their exercise performance, improved hormonal status, and energy metabolism in liver and skeletal muscle and tissues under the influence of whole-body vibration. The *P. scabiosifolia* tincture possesses a vibroprotective effect and can be recommended for the prevention and treatment of neurophysiological disorders caused by whole-body vibration, in combination with basic therapy.

Keywords: *Patriniascabiosifolia* Fisch. exLink .behavioral reactions, exercise performance, metabolism, whole-body vibration

АННОТАЦИЯ

Целью исследования была оценка эффективности *п. скабиозолистной* в коррекции физиологического и психосоматического состояния мышей, подверженных действию острой и длительной общей вибрации. Эффект 40%-ной водно-спиртовой настойки из корней *п. скабиозолистной* исследовался на мышах линии CD-1 при воздействии общей вибрации, индуцированной шуттель-встряхиванием или дорожно-транспортным воздействием. Эмоциональный, исследовательско-ориентировочный и двигательный компоненты поведения мышей наблюдали в «открытом поле». Физическую работоспособность оценивали по длительности плавания животных с 7%-м грузом на основании хвоста до полного утомления. Метаболическими показателями служили кортикостерон сыворотки крови, гликоген, лактат и аденозинтрифосфат печени и скелетной мышцы. Перорально введенный препарат в дозе 5 мл/кг оптимизировал поведенческие реакции животных, повышал их физическую работоспособность, улучшал гормональный статус и энергетический метаболизм в тканях печени и скелетных мышц и мышей при воздействии общей вибрации. Настойка *патринии скабиозолистной* обладает вибропротективным действием и может быть рекомендована для профилактики и лечения нейрофизиологических нарушений, вызванных общей вибрацией, в сочетании с базисной терапией.

Ключевые слова: *Patriniascabiosifolia* Fisch. *exLink*, поведенческие реакции, физическая работоспособность, метаболизм, вибрация

INTRODUCTION

Vibration is an extreme factor of the man-made environment, which to some extent accompanies man and animals: vibro-dangerous professional activity and all types of transportation, conditions of industrial livestock. To date, the influence of vibration on the physiological, neuroendocrine, morphological and metabolic levels of the body is fully studied; the concept of 'vibrational stress' and an independent nosological unit 'vibration disease' appeared in practical medicine and veterinary medicine (Babanov *et al.*, 2012). Under vibration stress (the initial and moderately pronounced manifestation of vibration in the early stages of the pathological process), the basic role in the interaction between various systems of the organism is played by the autonomous, peripheral and central nervous systems that trigger the subsequent psychosomatic disturbances (Ivanova, 1991; Ronchese and Bovenzi, 2012).

In spite of the fact a significant amount of measures funds aimed at eliminating various 'aggressive' effects of vibration are developed, fundamental and practical pharmacology aims at searching for and studying the action mechanism of known and recently emerged vibroprotectors (Johanning, 2015). In case of functional disorders of the nervous system and the somatic state of

humans and animals, adaptogens, anxiolytics (tranquilizers), plant-derived sedatives (Peeters *et al.*, 2005; Hur and Lee, 2010) are effective in a stressful situation.

The purpose of the study was to evaluate the effectiveness of the tincture of *P. scabiosifolia*, having adaptogenic and sedative action, as a vibroprotective agent.

MATERIALS AND METHODS

Sexually mature male mice of the CD-1 line (the nursery of the Pacific Institute of Bio-organic Chemistry, FER RAS) with a body weight of 22-25g were used in the study. The animals were kept in standard conditions of the vivarium; mixed feed compliant with GOST R 50258-92 (CJSC ProKorm, Russia) and water was supplied without restriction. Each experimental group consisted of 7 animals; each mouse in the cage had a floor area of 70 cm², which corresponds to international standards. The work was carried out in accordance with the normative documents 'Order of the Ministry of Healthcare of the Russian Federation No. 199n of April 1, 2016 On Approval of the Rules for Appropriate Laboratory Practice' and Directives 2010/63 / EU of the European Parliament and the Council of the European Union for the Protection of Animals used for scientific goals'. The tincture of *Patrinia*

scabiosifolia Fisch. ex Link (fam., Caprifoliaceae; TPS) was prepared by maceration from the roots and rhizomes in 40% ethanol (Vorobieva and Shabanov, 2015). In laboratory experiments, animals received a dealcoholized drug intragastrically at a dose of 5 ml/kg on an empty stomach in two ways: once an hour before the vibration (1TPS) or for five days preceding it (5TPS). In full-scale experiments, the drug was administered an hour before the road transportation. The animals of the control group and those exposed only to vibration (without the drug) received the equivolume amount of saline.

In laboratory conditions, whole-body vibration was simulated by placing the cell with mice on the platform of the ABU-6 shaker (oscillation frequency range 120 vibrations/min, acceleration 1.5 m/s², duration of a single exposure 2 hrs). The noise level created by the shaker was minimal and did not exceed 40 dB (measured by the NTM-1 sound level meter, Italy).

The state of physiological functions in mice during vibration was estimated by forced swimming test and behavioral activity tests. To determine exercise performance immediately after the two-hour vibration impact, mice were swimming with a 7% load of its body weight on the tail root until complete exhaustion in an 80 × 60 × 50 cm aquarium at a water temperature of 30 °C. Free behavior of the animals was assessed during 3 min in the open field test (installation 'Open Field', LLC Open Science, Russia) for the following components: horizontal motor activity (HMA; number of crossed arena sectors), vertical motor activity (VMA, standing alone or holding onto the installation wall), grooming (all types of body washing and cleaning), examination of the field holes, and the level of defecation and urination.

Biochemical indices were determined by unified methods: the level of corticosterone in the blood serum was measured spectrofluorometrically, the amount of lactate and adenosine triphosphate (ATP) in the liver and in the skeletal (gastrocnemius) muscle was measured spectrophotometrically (in the test system with nicotinamidedinucleotides NADP and NAD•H, respectively) and glycogen was measured using an anthrone reagent.

In the field experiment, the mice were subjected to single-day or seven-day road transportation along a highway with a hard surface, covering a distance of 100 km with an

average speed of 50 km/h. External weather conditions for transportation were as follows: clouds, air temperature 21 °C, air humidity 65%, atmospheric pressure 756 mmHg, the noise level on the highway 85-90 dB. Inside the bus cabin the temperature, humidity, the degree of dust and gas contamination corresponded to the norm, the noise level varied within 75-78 dB, the actual equivalent corrected levels of total vibration along the axes were: X-106, Y-105, Z-113 dB. To measure the level of vibration and noise in the cabin during the entire route, an on-site inspection was carried out in the testing laboratory of the Far Eastern Regional Center for Occupational Safety (Vladivostok) with the noise and vibration analyzer "Assistant".

Statistical processing was carried out with the program Statistica, v. 8.0. The significance of the differences between the compared groups was established using the Student's t-test and the one-way ANOVA dispersion analysis. The data in the tables are presented as the mean ± standard error of the mean (M ± m).

RESULTS

A two-hour stay in conditions of general vibration (transmitted through the supporting surfaces of the body) in a shaker caused in mice significant deviations in the body from the physiological norm in such vital functions as metabolism, physical performance, and behavioral responses. Table 1 presents the data on the significant disorders in the hormone-metabolic homeostasis of mice exposed to vibration. The animals showed marked stress, as indicated by the corticosterone level which exceeds the norm by 29%. In accordance with the regulatory role of corticosteroids in the metabolic systems of the body, there are significant deviations in the energy supply of the liver and skeletal muscle in mice under the impact of vibration. Immediately after vibration, these tissues displayed a clear deficit of energy substrates. Thus, the level of ATP and glycogen in the liver was 21 and 22% below the physiological norm, in the muscle, the indices were 22 and 29%, respectively. Moreover, both tissues revealed an increased content of lactate – 26 and 29%, respectively.

Patrinia, administered once or for five days running (once daily) before the vibration impact significantly minimized the degree of stress without changing the direction of the

hormonal and metabolic changes in the body (Table 1). The decrease in the level of corticosterone (stress marker) in the blood serum by 13 and 26% relative to the 'vibration' group (1TPS and 5TPS administered respectively) suggests optimizing the energy supply of the animal organism under the conditions of this unfavorable physical factor. Indeed, the content of ATP, glycogen and lactate in the liver differed from the norm only by 8, 6, and 9% (1TPS) and 3, 9, and 12% (5TPS), whereas in animals that did not receive the drug the deviations in case of vibration were 21, 22, and 26%, respectively. In skeletal muscle, a higher level of energy reserves was also observed: the difference compared to control in the levels of ATP, glycogen, and lactate was 11, 12, and 9% (1TPS) and 5, 8, and 14% (5TPS); in the 'vibration' group the levels were 22, 29, and 29%, respectively. The energy-protective effect of *Patrinia* is directly indicated by the conservation of the resources of ATP and glycogen in the liver and muscle tissues of mice at the optimal level with the simultaneous weakening of acidosis.

The study revealed that vibration significantly reduced the physical efficiency of mice. The duration of swimming time until complete fatigue after vibration was significantly lower than in the animals of the control group - by 31% (Table 2). The drug administered to the animals in both groups before the vibration increased the endurance during physical exertion by swimming. The endurance of the animals which were given TPS immediately after the exposure to vibration was below the control value only by 15 (1TPS) and 11% (5TPS); without the drug being administered the level was 31%, which indicates the actoprotective effect of TPS (Table 2).

In a parallel experiment, functional disorders of the nervous system in mice caused by general vibration were revealed. Analysis of motor activity in the open field showed a behavioral change in the animals, indicating their anxious excitement (Table 2). Horizontal motor activity in the open field (jogging through the arena sectors) 3 min after the vibration impact was above the control level by 52%. Vertical motor activity (standing) exceeded the norm by 21%. Acts of grooming (body washing and cleaning) characterizing the emotional state of animals were higher than the control level by 30%. It should be noted that defecation and urination (which are a manifestation of emotions) during the testing of mice in the open field were

practically not observed. However, in the cell where the animals were subjected to vibration boluses and wet zones were significantly increased in number than in the control group. The animals' research reaction (peeking into holes and sniffing) in the case of short (3 min) post-vibration testing was not observed, although the number of standing does reflect some research activity, in addition to nonspecific excitability. *Patrinia* revealed a neuroprotective effect, reducing emotional tension and bringing the correlation of behavioral responses into correspondence (Table 2). The drug is administered, HMA, VMA and grooming acts differed from the norm levels: in the case of 1TPS the levels were 22, 11 and 0%, and in the case of 5TPS the levels were 8, 15, and 10% respectively (in the control group the difference was 52, 21, and 30%).

In the field experiment, the mice were subjected to single-day or seven-day transportation (vibration combined with an inadequate noise level) along with a highway with a hard surface, covering a distance of 100 km. The physiological state of animals was judged by physical performance – an integral indicator of functional preparation for overcoming the effects of a negative stress factor. After single-day transportation, the ability of mice to swim until fully exhausted was reduced by 31%, and after seven-day transportation by 20% (Table 3). It should be noted that the physical capacity of animals with repeated exposure to vibration and noise is noticeably higher than in single exposure; apparently, there is some adaptation to this combined stressor. *Patrinia* tincture contributed to slower fatigue while performing intense unavoidable activity: the physical performance of mice in the 'forced swimming' test significantly exceeded the indication of the 'vibration' group subjected to single-day or seven-day transportation (vibration impact) - by 18 and 17%, respectively (Table 3).

DISCUSSION

The pattern of psychosomatic disorders in mice in the present experiment is consistent with various studies (Yanshina and Liubchenko, 2001; Mazina *et al.*, 2004). Deficiency of ATP, acidosis, and glycogenolysis in the liver and skeletal muscle indicate disruption of adaptive-compensatory capabilities in animals under

vibration impact. It is known that vibration (both local and general) act in combination with a number of environmental factors and dominates in technogenic conditions of animal life and in all types of transportation (Prisby *et al.*, 2008). Vibroacoustic effect on mice is stressful, as phylogenetically anthropogenic noise and vibration were absent in their vital natural activity. It should be noted that the noise and vibration levels in the cabin of the bus corresponded to the indications for the driver (GOST 12.1.012 - 2004 'SOSS (System of occupational safety standards), Vibration Safety: General Requirements'.

Metabolic and functional disorders in mice due to vibrational impact are a consequence of a neuro-reflex and neurohumoral nature (Toshima and Endo, 2006; Mazina *et al.*, 2004). With excessive excitation of the nervous system, which provokes psychosomatic disorders, anxiolytics, tranquilizers, and sedatives are used (Panossian and Wikman, 2005).

It is known that drugs based on *P. scabiosifolia* possess sedative and adaptive effect and are promising in arresting the excited state of animals (increased anxiety and motor hyperactivity) and, at the same time, disadaptation disorders from internal organs and tissues caused by a stressful situation and used in the treatment of insomnia, anxiety and inflammation of various genesis (Khasina, 2013; Meoli *et al.*, 2005; Zorikova and Khasina, 2005; Zou *et al.*, 2015).

The finding of the present study has shown that tincture *P. scabiosifolia* did not fully eliminate, yet it significantly reduced the degree of psychosomatic disorders in mice after a situational and prolonged vibration impact, apparently through the regulation of the neuroendocrine and metabolic links of the adaptive process.

P. scabiosifolia can be vicarious with *Valeriana officinalis* since it belongs to the same family of *Caprifoliaceae* (Hidalgo *et al.*, 2010). It is widespread in the Far Eastern region of the Russian Federation, northern China, Japan, and the Korean peninsula.

CONCLUSIONS:

The present research revealed that the *P. scabiosifolia* root tincture was efficient in suppressing vibration stress responses in mice.

This vibroprotective effect might be due to increasing duration of physical work until absolute fatigue and normalization of vibration-related behavioral alterations. This drug has increased the nonspecific resistance of mice to whole-body vibration by energy-saving effect at the expense of hindrance of exhaustion of glycogen and adenosine triphosphate reserves in the liver and skeletal muscle and optimization hormonal status of animals.

The findings of the present study indicate that *P. scabiosifolia* root tincture is promising as a preventive remedy for increasing the non-specific resistance of animals and human to whole-body vibration.

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Table 1. Influence of the tincture of *P. scabiosifolia* (TPS) on hormone-energy metabolism in mice under vibration

Parameter	Group of animals			
	control	vibration	vibration + 1TPS	vibration + 5TPS
Corticosterone, $\mu\text{mol/L}$	0,31 $\pm$ 0,02	0,40 $\pm$ 0,02*	0,35 $\pm$ 0,02	0,32 $\pm$ 0,01**
ATP, $\mu\text{mol/g}$				
liver	3,36 $\pm$ 0,07	2,65 $\pm$ 0,12*	3,08 $\pm$ 0,05**	3,26 $\pm$ 0,07**
muscle	4,40 $\pm$ 0,09	3,45 $\pm$ 0,13*	3,93 $\pm$ 0,07**	4,17 $\pm$ 0,10**
Glycogen, $\mu\text{mol/g}$				
liver	234,2 $\pm$ 11,1	181,8 $\pm$ 7,6*	220,2 $\pm$ 14,4**	213,5 $\pm$ 10,1**
muscle	23,5 $\pm$ 1,23	16,6 $\pm$ 1,04*	20,6 $\pm$ 0,71**	21,6 $\pm$ 0,74**
Lactate, $\mu\text{mol/g}$				
liver	2,40 $\pm$ 0,09	3,02 $\pm$ 0,10*	2,51 $\pm$ 0,17**	2,68 $\pm$ 0,10**
muscle	2,91 $\pm$ 0,14	3,75 $\pm$ 0,13*	3,17 $\pm$ 0,09**	3,33 $\pm$ 0,07**

Note: ATP – adenosine triphosphate; 1TPS – one-time introduction 1 hour before the vibration; 5TPS – an introduction for 5 days prior the vibration (once daily, on an empty stomach). * - $P < 0.05$ – compared with the 'control' group; ** - $P < 0.05$ – compared with the 'vibration' group.

Table 2. Influence of TPS on exercise performance and behavioral reactions of mice under the influence of whole-body vibration

Group of animals	Exercise performance		Locomotor activity acts		
	min	%	HMA	VMA	Grooming
control	20,4 $\pm$ 1,57	100	25,7 $\pm$ 2,40	7,57 $\pm$ 0,48	2,86 $\pm$ 0,26
vibration	14,0 $\pm$ 1,45*	69	38,9 $\pm$ 2,80*	9,14 $\pm$ 0,51*	3,71 $\pm$ 0,28*
vibration +1TPS	17,4 $\pm$ 0,10	85	31,3 $\pm$ 1,41**	8,43 $\pm$ 0,37	2,85 $\pm$ 0,26**
vibration +5TPS	18,1 $\pm$ 1,02**	89	27,6 $\pm$ 2,59**	8,71 $\pm$ 0,47	3,14 $\pm$ 0,26

Note: HMA – horizontal motor activity; VMA – vertical motor activity; 1TPS – the one-time introduction of *Patrinia* tincture; 5TPS – five-time one-time introduction of *Patrinia* tincture. * - $P < 0.05$ – compared with the 'control' group, ** - $P < 0.05$ – compared with the 'vibration' group.

Table 3. Influence of the TPS on the exercise performance of mice under the influence of road transportation

Group of animals	Road transportation			
	single		multiple	
	min	%	min	%
control	22.8 $\pm$ 1.3	100	19.4 $\pm$ 1.1	100
vibration	15.8 $\pm$ 1.2*	69	15.6 $\pm$ 0.9*	80
vibration+TPS	19.8 $\pm$ 1.3**	87	18.9 $\pm$ 0.9**	97

Note: TPS – tincture of *P. scabiosifolia*, * - $P < 0.05$ – compared with the 'control' group, ** - $P < 0.05$ – compared with the 'vibration' group.