

# A Dietary Supplement Based on Vegetable Oil Enriched With Altai Wapiti Velvet Antlers and Having Specific Functional Properties

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## ABSTRACT

This study investigated the effects of a dietary supplement containing lipids extracted from Altai wapiti antlers on patients with panic attacks (PA). Two groups of 30 patients aged 18-55 years were studied: the main group received the supplement, while the control group did not. Clinical effectiveness was assessed using questionnaires, adaptive response types, heart rate variability (HRV), and cytokine production. Results showed a significant decrease in PA manifestations, normalization of the autonomic nervous system function, and improved adaptive reactions in the main group. HRV analysis revealed a reduction in sympathetic tone and centralization of regulation processes. Hormonal status assessment indicated normalization of stimulated insulin and cortisol levels. The supplement also contributed to the normalization of spontaneous and PHA-stimulated production of IFN $\gamma$ , IL-2, IL-4, and IL-1 $\beta$  cytokines, which play a crucial role in the interaction between the nervous and immune systems, ensuring stress resistance and body adaptation. During the 2-week follow-up, the main group experienced fewer and less severe PA episodes compared to the pre-treatment period. The study confirms the effectiveness of the dietary supplement in the prevention and treatment of PA, highlighting its multiple actions on stress resistance, autonomic nervous system function, stress-regulating hormones, and cytokine balance.

**Keywords:** Dietary supplement, Vegetable oil enriched, Panic attacks.

## 1. INTRODUCTION

The modern period of human development is accompanied by significant changes in body physiology. This is due to the rapid development of civilization and its consequences, such as:

- imbalance in the diet in terms of essential nutrients and energy
- chronic deficiency in essential nutrients and the spread of alimentary diseases;
- industrial revolution with the development of artificial intelligence and digital technologies, automation, and computerization of production processes and living conditions
- emotional, and environmental stress that require quick and high-quality adaptation
- viral attacks in the era of COVID, which has a negative impact on the functioning of the central links of the body's regulatory systems: nervous, hormonal, immune, etc.

Particular attention is paid to the growth of functional disorders of the autonomic nervous system (ANS), including panic attacks (PA) [1-7]. A panic attack or autonomic crisis is an extreme form of vegetative-vascular dystonia, the main symptoms of which are panic disorders, nervousness, and feelings of unreasonable fear and anxiety [8-11]. The term PA is officially included in the International Classification of Diseases, 10th revision (ICD-10) under F 43.3 code. Approximately 40% of people who initially seek medical help from general practitioners have symptoms of PA. At the same time, the main age category is patients of working age, which indicates the relevance of the problem under consideration and the need to solve it [12-19].

The best way to overcome panic attacks is scientifically based on a combination of drug and non-drug therapies. The latter should include the use of adaptogens, a pharmacological group of substances of natural origin, one of which is Altai wapiti antlers. They can increase the body's non-specific resistance to a wide range of harmful

chemical, physical, and biological effects [20-28].

## 2. MATERIALS AND METHODS

Tests of a new dietary supplement based on vegetable oil containing lipids (phospho- and glycolipids) obtained by extraction from the Altai wapiti antlers were conducted. Two groups of patients were taken: main and control groups of 30 people aged 18–55 years. Ten drops of the dietary supplement were administered orally sublingually once a day before or during meals in the first half of the day for four weeks. The control group received no dietary supplements. Clinical studies were conducted based on the criteria for effectiveness immediately after the end of the diet therapy course. The observation lasted 2 weeks regarding the factors of recurrence of panic attack episodes.

The state of vegetative tone was determined using a quantitative (score) assessment of the presence of vegetative dystonia syndrome (Bain A.M., 2003. "Research schemes for identifying signs of vegetative disorders"). The clinical effectiveness of dietary supplements was studied by analyzing the main symptoms of a panic attack using a questionnaire. The assessment was carried out before and after four weeks of taking the functional product. Verification of the diagnosis of vegetative-vascular dystonia of manifested by panic attacks was carried out in patients by determining the presence of at least four symptoms.

The type of adaptation response was determined by the percentage of lymphocytes in the white blood cell count and their ratio to segmented neutrophils by the method introduced by Golberg et al. (1996).

The study of heart rate variability (HRV) was conducted by recording short fragments of a standard cardiointervalography with a hardware and software complex.

When analyzing cardiointervalography, the following indicators were assessed: stress index; heart rate; heart rate variation characterizing the degree of influence of the parasympathetic division of the autonomic nervous system on heart rhythm (HR); mode reflecting the state of the humoral regulation channel; the index of centralization reflecting the degree of centralization of heart rhythm control, considering the state of the humoral regulation channel and the activity ratio of the parasympathetic and sympathetic divisions; mode amplitude showing the degree of influence of the sympathetic division on the heart rate (HR).

The level of the main stress-resistant cytokines IFN $\gamma$ , IL-2, IL-4 and IL-1 $\beta$  in the supernatants was determined by the solid-phase sandwich enzyme immunoassay method consisting in adsorption on the solid phase of the microplate (the procedure was carried out according to the guidelines by Procon (Russia) and CytImmune (USA)). The studies were carried out at the Scientific and Clinical Center for Hormonal Health ProfMed at the Obstetrics and Gynecology Department of the Siberian State Medical University (Tomsk) under the supervision of Doctor of Medical Sciences, Professor L.S. Sotnikova.

## 3. RESULTS AND DISCUSSION

During screening, significant disorders of the autonomic nervous system were observed in all patients. The total score in the main group with panic attacks did not correspond to the standard indicators (below 10 points) and averaged  $85.3 \pm 5.05$ , which exceeded the standard by more than 8 times [29-34]. The subjects in the main group exhibited an average of  $10 \pm 2$  clinical manifestations of a panic attack. Table 1 presents the results of the study on the number of patients with clinical manifestations of panic attacks before and after dietary supplementation.

**Table 1.** Clinical manifestations of panic attacks before and after taking the dietary supplement (DS) (number of patients who exhibited this manifestation).

Clinical manifestations of panic attacks	DS Before treatment n=30	DS Taken for 4 weeks n=30	Control Group n=30
Shakiness inside, shaking in the hands, the chills	28	5*	0
Excessive sweating, cold sweat	14	3*	0
Fast pulse, racing heartbeat	22	6*	0
Stomach discomfort, nausea	19	8*	0
Chest pain or discomfort	28	10*	0
Difficulty breathing, shortness of breath	19	4*	0
Fear of performing some act, of going crazy	12	2*	0
Feeling detached from reality, the world doesn't feel real	10	2*	0
Feelings that you're seeing yourself from the outside, that you have no control over yourself	26	0*	0
Dizziness, feeling off balance, fainting	24	3*	0
Insomnia	30	8*	0
Unpleasant sensations in the hands and feet: coldness, pins-and-needles, numbness	22	4*	0

Difficulty concentrating or thinking clearly	30	5*	0
Fear of death	28	0*	0

Note: \* reliable differences ( $p < 0.05$ ) compared to "before treatment".

Analysis of changes in vegetative-vascular dystonia manifested by panic attacks showed a reliable ( $p < 0.05$ ) decrease in clinical manifestations of panic attacks in all patients taking the dietary supplement. It is important to note the complete relief of such manifestations as fear of death and the feeling of inability to control oneself at a given time [35-40].

Complaints related to the prescription of the dietary supplement were not identified, side effects were not registered. During the survey of patients, a subjective improvement in well-being was established manifested by a decrease in emotional lability, an increase in vitality and performance, the effectiveness of work, less complaints about panic attacks in all patients.

A comprehensive in-depth assessment of the functional state of the adaptive potential of the autonomic nervous system was carried out (Table 2).

**Table 2.** Distribution of adaptive reactions in patients before and after taking the functional product ( $X \pm m$ , p).

Adaptive Response	DS Before treatment n=30	DS Taken for 4 weeks n=30	Control Groupn=30
Training and activation responses	0	18*	30
Acute stress	14	2*	0
Chronic stress	16	10*	0

Note: \* - reliable differences ( $p < 0.05$ ) compared to "before treatment".

The course use of dietary supplements in patients with PA led to a significant redistribution of adaptive response types towards a reliable increase in the number of patients with a physiological response of training and activation, as well as a reliable ( $p < 0.05$ ) decrease in the number of patients with acute and chronic stress [41].

Kérdő Autonomic Index (KAI) in patients averaged  $44.2 \pm 2.32$  conventional units, indicating a pronounced predominance of excitatory effects in the activity of the autonomic nervous system. The predominance of sympathetic tone causes tension in the adaptive capabilities of the body, which is clinically manifested by the presence of symptoms of autonomic irritation and panic attack episodes, which were identified during anamnesis procedure and the questionnaire survey of the subjects [42].

When assessing the parameters of the heart rhythm based on the results of cardiointervalography, all patients showed an increase in the tone of the sympathetic division of the ANS and a decrease in parasympathetic regulation, which was expressed by a statistically significant increase in the mode amplitude and the stress index, as well as the variation range (in comparison with normal values). The maximum sympathicotonia was recorded in 35 to 45 age group. The data obtained confirm the imbalance of ANS divisions manifested by pronounced sympathicotonia, centralization of regulation processes and a decrease in adaptive mechanisms [43].

It was concluded that diet therapy represented by the intake of dietary supplements leads to reliable normalization of the functional state of the autonomic nervous system, increased overall performance and adaptive reactions. When analyzing the severity of psychopathological symptoms (using psychometric assessment of mental and emotional disorders using diagnostic scales), positive dynamics ( $p < 0.05$ ) of the state of the central and autonomic nervous systems was observed, which was manifested in the normalization of the psychoemotional background and a significant reduction of asthenic symptoms [44,45]. The current functional state of half of the patients was close to satisfactory - 8-10 points ( $p < 0.05$ ). The reactivity of the sympathetic division of the ANS during the orthostatic test was significantly reduced ( $p < 0.05$ ). Vegetative support of activity in functional tests was characterized by adequate activation of the parasympathetic division of the ANS. The mechanism of harmonization of the autonomic nervous system is associated with a decrease in tone through the sympathetic nervous system and its normalization under the influence of the parasympathetic nervous system. Table 3 shows the influence of the nutritional factor on the functional state of the autonomic nervous system.

**Table 3.** Indicators characterizing the state of the autonomic nervous system in patients before and after taking the functional product ( $X \pm m$ , p).

Parameter	DS Before treatment n=30	DS Taken for 4 weeks n=30	Control Groupn=30
KAI, relative unit	$44,24 \pm 2,32$	$12,28 \pm 0,02^*$	$2,23 \pm 0,04$

Mode, sec.	0,80±0,13	1,98±0,12*	2,46±0,27
Mode amplitude, %	78,27±3,01	40,10±1,25*	38,14±2,11
Variation range, sec.	1,10±0,05	0,22±0,04*	0,28±0,02
Stress index, relative unit	223,24±4,08	124,35±2,05	138,23±5,12

Note: \* - reliable differences ( $p < 0.05$ ) compared to "before treatment".

The study of the heart rate variability as a way to assess the state of vegetative support of functions, was carried out 4 weeks after prescription of the dietary supplement. According to relevant studies, such a period is quite sufficient to stop vegetative reactions in the form of panic attacks, which is confirmed by the correction of vegetative dysfunction with diet therapy.

A comprehensive analysis of hormonal status parameters revealed statistically significant ( $p < 0.05$ ) trends in the normalization of stimulated insulin and cortisol levels when using the dietary supplement ( $p < 0.05$ ). No changes in the levels of thyroid hormones and prolactin were detected.

Normalization of the stimulated insulin level is a consequence of the normalization of the cortisol level under the influence of the functional product, since it has adaptogenic properties and neutralizes the pathological influence of the sympathetic nervous system. As a result, the cortisol level and, as a consequence, insulin is normalized due to insulin-stimulating effect, indicating a positive effect of the nutritional factor on stress-dependent hormones (Table 4).

**Table 4.** Hormonal status in patients before and after taking the dietary supplement ( $X \pm m$ , p).

Parameter	DS Before treatment n=30	DS Taken for 4 weeks n=30	Control Group n=30
Basal insulin (mU/ml)	12 [8:14]	10 [8:16]	2,7 – 10,4
Stimulated insulin (mU/ml)	32 [22:40]	14* [8:18]	2,7 – 10,4
Cortisol (nmol/l)	840	550*	150 - 650
Prolactin (mU/l)	590 [500:600]	535 [420:550]	109 – 557
TSH (mU/l)	3,6 [2,6:3,8]	3,4 [2,8:3,6]	0,4 – 4,0

Note: \* - reliable differences ( $p < 0.05$ ) compared to "before treatment".

The association between the biological correlation of the stress response and somatic complaints was studied before the appearance of clinically expressed psychopathological disorders, such as panic attacks. Early predictors of vulnerability to somatization are increased blood cortisol levels and decreased heart rate variability. These changes significantly reduce the body's resistance to the effects of typical psychosocial stressors.

The relationship between manifestations of the mental sphere, nervous, endocrine and immune systems has been established, which is the basis for the functioning of the psychoneuroimmunoendocrine system. Almost all immune cells have receptors for hormones associated with the sympathetic-adrenal axes, called stress hormones. Immune modulation with the help of these hormones can be carried out in two ways: directly, by binding to their receptors on the cell surface or indirectly, inducing dysregulation of the production of cytokines, which have many functions and effects on the metabolism of the main neuropeptides and neurotransmitters (tryptophan, serotonin, norepinephrine, dopamine, GABA, etc.). It has been established that the balance between cytokines plays an important role in modulating cellular reactions in the brain during psychological stress and mental disorders, including panic attacks.

Thus, cytokines are involved in intercellular interaction and regulation not only of the immune system, but also of the hormonal and nervous systems. Cytokine balance has a significant impact on the psycho-emotional status. Evaluation of the effect of the studied dietary supplement on the cytokine profile is important for substantiating the mechanisms of its action for panic attacks.

Table 5 presents the results of spontaneous and PHA-stimulated production of cytokines by Th1 and Th2 lymphocytes in peripheral blood.

**Table 5.** Spontaneous and PHA-stimulated production of cytokines by Th1 and Th2 lymphocytes in peripheral blood ( $X \pm m$ , p).

Recorded Indicator		Observation groups		
		DS prior to treatment n=30	DS taken for 4 weeks n=30	Control Group n=30
IL-1 $\beta$	Spontaneous, pg/ml	12128±6,87	79,97±532 ( $p < 0,05$ )	50,91±3,09

	PHA-stimulated, pg/ml	95,46±532	103,46±3,09	132,87±5,97
	Stimulation Index	2,25±0,02	2,29±0,03	238±0,05
IL-2	Spontaneous, pg/ml	2325±3,91	52,02±1,15 (p<0,05)	46,70±3,65
	PHA-stimulated, pg/ml	105,63±4,02	152,09±5,02 (p<0,05)	19630±8,42
	Stimulation Index	1,98±0,02	5,52±0,03 (p<0,05)	438±0,08
IL-4	Spontaneous, pg/ml	11633±5,92	7933±3,79 (p<0,05)	58,89±334
	PHA-stimulated, pg/ml	23631±11,02	193,23±7,02 (p<0,05)	146,05±624
	Stimulation Index	2,03±0,02	2,46±0,03 (p<0,05)	2,48±0,03
IFN $\gamma$	Spontaneous, pg/ml	116,82±7,09	179,82±5,15 (p<0,05)	206,01±5,78
	PHA-stimulated, pg/ml	123,09±5,57	207,42±624 (p<0,05)	25632±8,09
	Stimulation Index	0,97±0,02	1,14±0,03	2,61±0,02

Note: reliable differences ( $p < 0.05$ ) compared to "before treatment".

Activation of Th1 lymphocytes, associated with the production of key cytokines IL-1 $\beta$ , IL-2 and IFN $\gamma$ , enhances cellular immunity. Determination of the humoral immune response is carried out with the dominant influence of Th2 cytokines, IL-4 being the discriminant one. The study of the cytokine-producing function of blood mononuclear cells in the group with PA revealed the change in the studied parameters characterizing the ability of immunocompetent cells to release cytokines.

The study of the level of mononuclear IL-1 $\beta$  synthesis of showed a significant increase in its spontaneous release in patients with panic attacks and a decrease after the use of the dietary supplement to values close to those of the control group. PHA-stimulated production of the studied interleukin and the stimulation index were at a comparable level in all groups [46].

All subjects with panic attacks showed suppression of spontaneous and PHA-stimulated production of IL-2 discriminant immune response in the implementation of the Th1 pathway. The cytokine secretion index in this group was significantly lower than in the control groups. The use of diet therapy restores the production of IL-2 cytokine and brings its levels closer to the control values. The significant increase was noted in basal and PHA-stimulated production of IL-4 by mononuclears, stimulating the polarization of T-helpers along the Th2 pathway. After terminating the intake of the dietary supplement, the secretion of the cytokine by immunocompetent cells was restored, but still remained significantly higher than the control values [47].

It was found that spontaneous and induced production of IFN $\gamma$  by immunocompetent cells was lower than the control group values. This gives grounds to assume a low level of antiviral immune protection in patients with panic attacks. The use of dietary supplements restores IFN $\gamma$  synthesis by immunocompetent cells, however, it remains lower than the level in patients in the control group. It was concluded that the nutritional factor contributes to the normalization of spontaneous and PHA-stimulated production of IFN $\gamma$ , IL2, IL4 and IL1 $\beta$  cytokines, which have an important pathogenetic significance in the joint functioning of the nervous and immune systems, ensuring stress resistance and body adaptation.

After completion of the diet therapy course, dynamic observation of patients was carried out for 2 weeks to assess the frequency of recurrence of panic attack episodes. Panic attacks were noted in 12 of 30 patients. The degree of their severity was significantly less than before the use of the dietary supplement. Recorded panic attacks lasted from 10 to 20 minutes, on average 10±5 minutes. The frequency of attacks was significantly less than before the diet therapy course. Thus, the conducted studies reveal a multiple action of the dietary supplement taken as a course by patients with panic attacks, resulting in:

- 1) increased level of stress resistance and body adaptability;
- 2) normalized function of the autonomic nervous system by means of the reduced tone of its sympathetic division;
- 3) normalized levels of stress-regulating hormones - cortisol and stimulated insulin due to their reduced content;
- 4) ensured cytokine balance - the production of key cytokines that affect the functions and interrelation of the hormonal, nervous and immune systems.

The multiple action of the functional product leads to a reliable reduction in the severity, duration and frequency of panic attacks. The analysis of the results of clinical trials of the developed dietary supplement confirms its effectiveness for prevention and treatment of vegetative-vascular dystonia manifested by panic attacks.

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