Publication date: 01.12.2021
DOI: 10.51871/2588-0500_2021_05_04_1
UDC: 612.1/.8

THE EFFECTIVENESS OF USING MARAL VELVET ANTLERS IN THE CORRECTION OF IMMUNE DYSFUNCTION IN ATHLETES

S.V. Vereshchagina[1], I.N. Smirnova[2], N.G. Abdulkina[2], B.V. Barankin[1]
[1]Federal State Budgetary Institution "Federal Siberian Scientific and Clinical Center of the Federal Medical and Biological Agency", Krasnoyarsk, Russia
[2]Federal State Budgetary Institution "Siberian Federal Scientific and Clinical Center of the Federal Medical and Biological Agency", closed city Seversk, Russia

Key words: athletes, immune system, maral velvet antlers.

Annotation. The aim of the study is to scientifically substantiate feasibility of the use of maral velvet antler powder in correction of immune dysfunction in athletes of winter cyclic sports in the preparatory period of the annual training cycle. The conducted study proved that using maral velvet antler powder at a dose of 4 grams per day contributes to an increase in phagocytic and metabolic activity of leukocytes, correction of cytokine balance that allows to consider maral velvet antlers as an effective measure to correct immune dysfunction as one of the markers of overtraining syndrome in athletes of winter cyclic sports.

Introduction. Modern sports imply achieving the highest results that are possible only in conditions of performing excessive loads. An absence of adequate recovery in case if these states develop against the background of further physical and psychoemotional loads leads to worsening of pathological changes in the athlete's organism and development of the systematic maladaptation syndrome, which in the long run transforms into the overtraining syndrome [1-3]. Scope and amount of studies dedicated to the diagnosis and correction of signs of overfatigue and overtraining in athletes has been increasing in recent years [2-3]. Analysis of conducted research show that attention of sports experts in connection with tightened regulations of the Anti-Doping Agency is aimed primarily at the search for drug-free means of recovery, especially natural drugs, components of which are similar to those of the human's organism [4].

One of the most prosperous directions of non-drug support of the organism associated with intense physical loads is using adaptogenes, especially preparations made from products of the velvet antler industry, efficiency of which was proven in several conducted studies [4-6]. Using antler-based preparations in sports medicine

is mainly related to using pantohematogen – freeze-dried blood of a maral (Caspian red deer). Meanwhile, using powder of maral velvet antlers – the richest substance from the point of view of biological components – is still insufficiently studied.

The aim of the study is to scientifically substantiate feasibility of the use of maral velvet antler powder in correction of immune dysfunction in athletes of winter cyclic sports in the preparatory period of the annual training cycle.

Methods and organization. The work was conducted in the FSBI "Federal Siberian Scientific and Clinical Center of the Federal Medical and Biological Agency" (Krasnoyarsk) and the Tomsk Scientific Research Institute of Balneology and Physical Therapy, branch of the FSBI "Federal Siberian Scientific and Clinical Center of the Federal Medical and Biological Agency" (Tomsk) within the State Contract №44.001.11.14 of the "Biomedical and Medicosanitary Support of Athletes of the Russian Federation National Teams" targeted program of the FMBA of Russia.

Selection of athletes of winter cyclic sports took place in the Regional Center of Sports Training "Academy of Winter Sports" (Krasnoyarsk) and the "Biathlon Academy (Krasnoyarsk). At the clinical stage, 86 athletes of winter cyclic sports (ski racing, biathlon), aged 18-30 years (average age of 21,90±4,19 years) were examined according to the purpose and tasks of the study. Using the simple random sampling, we divided athletes into 3 groups matched by age, constitution features, level of athletic prowess and athletic experience:

- main group I (n=30) took the maral velvet antler powder in capsules at a dose of 2 g/day;
- main group II (n=30) took the maral velvet antler powder in capsules at a dose of 4 g/day;
- control group III (n=26) took placebo (powdered sugar in similar capsules) and did not take other supplements.

Intake of the powder and placebo took place at the preparatory stage; a course of administration was 14 days.

Clinical, laboratory and instrumental studies of athletes were carried out before and after the course of powder administration at doses of 2 g/day and 4 g/day during the preparatory (September-October) period of the annual training cycle.

Content of circulating immune complexes (CIC) in the blood serum was examined in the precipitation reaction with a polyethylene glycol solution. We evaluated the phagocytic component of the immune system of athletes in accordance with the non-specific phagocytosis examined with the method developed by V.M. Berman and E.M. Slavskaya (1958) with the Staph. Aureus (strain 209) testing culture. We also assessed the absorption capacity of neutrophils using calculation of the phagocytic index (PhI) – percentage of phagocytes from the

number of counted neutrophils, and the phagocytic number (PhN) – mean number of microbes absorbed by one phagocyte. PhI and PhN values were defined before (spontaneous) and after stimulation of phagocytes with prodigiosan (stimulated).

The metabolic activity of phagocytes was assessed according to their ability to recover nitro blue tetrazolium (NBT) in the spontaneous and stimulated NBT test. We registered a relative number of neutrophils that have diformazan deposition before and after stimulation with the S. marcescens vaccine in $2 \cdot 10^9$ microbial bodies in 1 ml (stimulated NBT test). The S. marcescens vaccine was created in the Kazan' Scientific-Research Center of Epidemiology and Microbiology and recommended as a special standard stimulator of such reaction.

The cytokine status was evaluated with pro-inflammatory (IL-1β, IL-6, TNF-α) and anti-inflammatory (IL-4) cytokines using the immunoenzymatic method with the Stat Fax 303 Plus® Microstrip Reader (Awareness Technology, USA) and "VektorBest" sets (Russia).

To carry out statistical processing of the data obtained during the study, we used the Statistics 8.0 software. The Shapiro-Wilk test was used to test normal distribution. In case if distribution differed from normal, we presented data in a form of "mean±standard derivation" (M±SD), for describing distribution we used median (Me) and interquartile range in a form of Me[LQ;UQ], where LQ is lower quartile, UQ – upper quartile. If a distribution of examined selections differed from normal, we used the Wilcoxon's T-test. In order to define significance of differences between independent selections, we used the Mann-Whitney U-test.

Results and discussion. Evaluation of dynamics of indicators of systemic immunity and non-specific resistance of the organism of athletes of winter cyclic sports during the preparatory period revealed following patterns. An ability of leukocytes to phagocytosis evaluated according to the PhI indicator was decreased in 80,2% (n=69) of athletes in all groups by the beginning of the study. Low PhN values were also registered in more than the half of athletes – 79,1% (n=68). The revealed changes of nonspecific resistance of the organism associated with intense physical loads can be a predictor of developing immunodeficiency and decreasing resistance to infectious diseases [2, 7].

Within the observation process, it was shown that among the control group athletes intense physical loads led to further decrease of both spontaneous (by 12%, p=0,014) and stimulated with prodigiosan (by 34%, p=0,001) phagocytic activity of leukocytes, as shown in a table below.

Administration of velvet antlers powder at a dose of 4 g/day contributed to an increase of the PhIspont by 32% (p=0,008) and PhIstim by 11,1% (p=0,033), as well as an increase of phagocytic cells of PhNspont and PhNstim by 12,5% (p=0,005) and 14,3% (p=0,049) respectively (table).

Table

Dynamics of immunological indicators in athletes associated with an intake of the maral velvet antlers powder (Me [LQ;UQ])

Indicators	Main group I, n=30	Main group II, n=30	Control group III, n=26
	before intake after intake	before intake after intake	before intake after intake
PhIspont, % (standard: 40-80)	45,0 [43,5; 50,0] 46,0 [40,0; 52,0] p=0,535	36,0 [26,5; 40,5] 47,5 [41,0; 51,0] p=0,008	41,0 [36,0; 49,0] 36,0 [29,0; 41,0] p=0,014
PhIstim, % (standard: 40-80)	39,5 [34,0; 45,0] 33,0 [24,0; 49,0] p=0,379	45,0 [41,5; 55,0] 50,0 [48,0; 54,0] p=0,033	50,0 [39,0; 56,0] 33,0 [28,0; 36,0] p=0,001
PhNspont, u. (standard: 4-9)	4,2 [3,4; 4,8] 4,2 [3,7; 4,4] p=0,955	4,0 [3,7; 4,2] 4,5 [3,9; 5,1] p=0,005	2,9 [2,6; 3,8] 3,5 [2,8; 4,0] p=0,244
PhNstim, u. (standard: 4-9)	3,8 [3,2; 4,3] 4,0 [3,2; 4,3] p=0,623	3,5 [3,2; 4,3] 4,0 [3,6; 4,3] p=0,049	3,3 [2,3; 4,6] 3,2 [3,0; 4,2] p=0,842
NBTspont, % (standard: 10-15)	24,5 [16,5; 28,5] 19,5 [16,0; 21,5] p=0,006	26,0 [21,5; 33,0] 21,5 [13,5; 24,5] p=0,016	17,0 [16,0; 27,0] 19,0 [17,0; 29,0] p=0,221
NBTstim, % (standard: 40-80)	33,0 [29,5; 36,0] 43,5 [36,5; 49,5] p=0,000..	47,5 [37,0; 61,5] 53,0 [45,0; 66,0] p=0,004	39,0 [32,0; 52,0] 29,0 [26,0; 32,0] p=0,004
CIC, u. (standard: 0-90)	63,5 [55,5; 71,5] 63,0 [60,5; 78,0] p=0,245	78,5 [62,0; 86,0] 62,5 [50,5; 74,5] p=0,000…	48,0 [42,0; 89,0] 58,0 [54,0; 70,0] p=0,064
TNF-α, pg/ml (standard: 0-6)	1,95 [1,60; 2,35] 1,85 [1,25; 2,05] p=0,006	2,00 [2,00; 2,50] 1,75 [1,35; 2,05] p=0,002	2,10 [2,10; 2,20] 2,10 [2,00; 2,30] p=0,730
IL-1β, pg/ml (standard: 0-11)	1,40 [1,05; 1,50] 1,05 [0,75; 1,45] p=0,013	1,55 [1,25; 2,15] 1,15 [1,10; 1,55] p=0,004	1,40 [1,30; 1,70] 1,20 [1,10; 1,30] p=0,069
IL-4, pg/ml (standard: 0-13)	1,25 [1,05; 1,45] 1,60 [1,50; 1,80] p=0,002	1,15 [1,00; 1,75] 1,70 [1,20; 2,60] p=0,025	1,30 [1,00; 1,60] 1,40 [1,20; 2,20] p=0,003
IL-6, pg/ml (standard: 0-10)	1,05 [0,60; 1,40] 1,00 [0,70; 1,20] p=0,480	1,15 [0,95; 1,45] 0,80 [0,65; 1,00] p=0,002	1,20 [1,10; 1,60] 1,20 [0,90; 1,30] p=0,158

The dose of 2 g/day in the I group appeared to be insufficient for stimulating the phagocytic activity. At the same time, there was no significant worsening of the state of nonspecific reactivity, as it was registered in the control group.

The NBT spontaneous test allows assessing the state of the oxygen-dependent mechanism of blood phagocytes' bactericidal activity in vitro and characterizes an activity of the NADPH oxidase antibacterial system. Increase in the spontaneous NBT test is registered in case of bacterial antigens' influence, allergic and autoimmune states, as well as an enhancement of the antibody-dependent

cytotoxicity of phagocytes. Decrease of antigenic loads is accompanied by the indicator's normalization. Rapid decrease of the spontaneous NBT test indicates a decompensation of anti-infectious protection and counts as a prospectively unfavorable sign [7].

In our study, most athletes (93%, n=80) were characterized with high values of the NBTspont, which indicated pronounced antigenic load on the one hand and an increase in metabolic activity of non-activated phagocytes on the other hand. A revealed decrease of spontaneous metabolic activity of non-activated leukocytes in the NBTspont in vitro can be considered as a sign of the anti-inflammatory effect of velvet antlers. However, by the end of the study values of the NBTspont remained higher than standard ones in all groups, which indicated preservation of phagocytic neutrophils' dysfunction from the point of their metabolic activity (table).

An ability of leukocytes to activate in the NBT test significantly differed in observation groups. Low values of the NBTstim (lower than 40%) show presence of hyporesponsiveness of neutrophils to the synthesis of reactive oxygen species (ROS) as a response to stimulation with Staph. Aureus and a decrease of the oxygen-dependent bactericidal function of neutrophils [7]. By the beginning of the study, a decrease of potential activity of leukocytes in the NBTstim was registered in 65,1% (n=56) of athletes. We also noted an increase in reserves of leukocytes' activation in the NBTstim in the I group by 31,8% (p=0,000…) and the II group by 11,6% (p=0,004) within limits of standard values, which indicated an increase of reserve capabilities of leukocytes.

Meanwhile, athletes of the control group III revealed a decrease of the ability of leukocytes to activate by 25,6% (p=0,004), which, alongside with a pronounced low phagocytic activity, can be considered as a predictor of developing infectious complications and immunodeficiency.

Taking into account the fact that the leukocyte system plays an important role in maintaining dynamic balance of the most various factors of immune protection, we carried out an analysis of some indicators of the cytokine status, i.e. TNF-α, IL-1ß, IL-6, IL-4 cytokines (table). As the results show, values of both pro- and anti-inflammatory cytokines in the preparatory period of the annual training cycle were within the range of standard values in most athletes, which is a prospectively favorable moment when it comes to a development of secondary immunodeficiency that limits the growth rate physical performance.

The analysis of dynamics of the cytokine content revealed an increase in the cytokine balance in form of a decrease in TNF-α, IL-1ß and IL-6 cytokines and an increase in the level of IL-4 cytokine in groups of athletes, who took maral velvet antler powder, which allows considering the preparation as an immune system

modulator aimed at a decrease of immune pathological responses appearing against the background of training loads.

The comparative analysis of CIC mean values' dynamics registered a decrease of their concentration in the blood serum by 20,4% (p=0,000…) in athletes of the II group, which indicates an effective elimination and a decrease of antigenic loads.

Conclusion. Therefore, using maral velvet antler powder contributes to an increase of the phagocytic activity of leukocytes, improvements in the humoral immunity and cytokine balance states, which in the long run determines an anti-inflammatory and immune modulating effect, implementation of which is a basis of preventing infectious complications and the "open window" syndrome in athletes of winter cyclic sports at the preparatory stage of the annual training cycle.

References

1. Andrianova E.Yu. Sports medicine: a textbook for universities / E.Yu. Andrianova // Moscow: Yurajt. – 2020. – 325 p.

2. Dikunets M.A. Analysis of hypotheses for the development of overtraining syndrome / M.A. Dikunets, G.A. Dudko, E.N. Shachnev // Sports Medicine: Science and Practice. – 2019. – Vol. 9. – № 2. – P. 5-14.

3. Nikulina G.Yu. Modern criteria of overstrain and hypotheses of overtraining syndrome in athletes / G.Yu. Nikulina // Applied Sports Science. – 2020. – № 1(11). – P. 98-105.

4. Litvin F.B. Complex application of natural biostimulants in the training process of highly qualified athletes / F.B. Litvin, T.M. Brook, P.A. Terekhov // Human. Sport. Medicine – 2018. – Vol. 18. – № S. – P. 135-139.

5. Guryanov Yu.G. Products based on pantohematogen: effect mechanisms and features of application in sports / Yu.G. Guryanov // "Sports medicine. Health and Physical Culture. Sochi 2011": materials of the II All-Russian Scientific and Practical Conference, June 16-18, 2011. – Sochi. – 2011. – P. 85-87.

6. Latkov N.Yu. Questions of nutrition in sports of higher achievements: a monograph / N.Yu. Latkov, V.I. Pozdnyakovskij // Kemerovo: Kemerovo Technological Institute of Food Industry. – 2016. – 215 p.

7. Yarets Yu.I. Interpretation of the immunogram results / Yu.I. Yaretz // Gomel': State Institution "RSPC RMHE". – 2020. – 38 p.

Information about the authors: Svetlana Viktorovna Vereshchagina – Head of the Laboratory Diagnostics Department of the FSBI FNCC FMBA of Russia, Krasnoyarsk, e-mail: vereschagina.sv@mail.ru**; Irina Nikolaevna Smirnova** – Doctor of Medical Sciences, Head of the Therapeutic Division of the Department of Prevention and Restorative Treatment of Occupational Diseases of the Branch of the TSRIBiP FSBI SibFSCC of FMBA of Russia, Seversk, e-mail:

irin-smirnova@yandex.ru**; Natal'ya Gennad'evna Abdulkina** – Doctor of Medical Sciences, Deputy General Director for Scientific and Clinical Work of the FSBI SibFSCC of FMBA of Russia, Seversk, e-mail: nauka@med.tomsk.ru**; Boris Vladimirovich Barankin** – General Director of the FSBI FNCC FMBA of Russia, Krasnoyarsk, e-mail: skc@fmbamail.ru.