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By-products of *Pinus pumila* (Pall.) Regel in adaptogenic functional foods

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Abstract:
Physical and psychological stress is part of modern life, which means that consumers need alimentary support to restore their functional reserves. This research aimed to develop a new functional snack bar with reliable adaptogenic properties based on secondary raw materials of dwarf cedar (*Pinus pumila* (Pall.) Regel).

The main objects of the study were pine nut cake and microstrobiles – waste products of pine nut oil production and pollen harvesting. Additional components included dried sea buckthorn cake, flaxseed cake, and date paste. The nutritional and bioactive profiles were determined by standardized methods. The adaptogenic properties were studied on azathioprine immunosuppression in laboratory mice (Open Field and Porsolt Forced Swim Tests). The cytomorphological examination relied on standard methods.

The snack bar developed had a high content of dietary fiber, essential amino acids, polyunsaturated fatty acids, vitamins, minerals, and polyphenolics. When introduced into the diet of mice, it levelled the negative impact of azathioprine and restored the initial indicators of general physical activity, exploratory behavior, endurance, anxiety, and relative weight of the liver. It also helped the laboratory animals to recover the healthy morphofunctional state and weight of the major immune organs, i. e., thymus and spleen. The fact that the mice regained their nonspecific resistance during immunosuppression was apparently associated with the antioxidant activity of the bioactive components in the experimental snack bar.

In this research, by-products of pine nut processing and pollen harvesting yielded a new functional product with reliable adaptogenic properties. Natural phytocomponents in mass-market products can facilitate consumers' adaptability to adverse environment by improving resistance to various stresses.

Keywords: Pine nut cake, *Pinus pumila*, microstrobiles, functional product, snack bar, adaptogenic properties, histomorphology, azathioprine, immunosuppression, mice

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INTRODUCTION

The 21st century with its informational, psychological, and emotional overload, aggravated by environmental pollution, has seen radical changes in human diet. These challenges have already changed the general morbidity and mortality patterns. Today, cardiovascular, oncological, and diabetic diseases of metabolic nature are responsible for most deaths. Food science sees it as its priority task to help people to adapt to the new stressors and develop protective mechanisms.

From a physiological perspective, adaptation is a set of physiological reactions that facilitate internal adjustments to changing environment, allowing the organism to survive and maintain vital activity in the face of new conditions [1, 2]. Adaptability relies on adaptogens, i. e., simple or complex substances that stimulate defenses

and support long-term health by stabilizing the internal environment. Natural adaptogens that make up a considerable part of traditional medicine are in the spotlight of contemporary food science. For instance, preparations based on *Panax ginseng* C.A. Mey., *Eleutherocóccus senticosus* (Rupr. et Maxim.) Maxim, *Rhodiola rosea* L., and *Schisandra chinensis* Turcz. (Baill.) are low-toxic and side-effect-free. They contain bioactive substances that provide a mild but long-lasting effect. These properties trigger long-term adaptation mechanisms that prevent diseases and develop resilience to bad environment. The search for new natural adaptogens continues as food science updates its knowledge about the positive effects of certain natural compounds on physical resilience.

Plant-based bioactive substances are mentioned in the Standards of Physiological Requirements for Energy and Nutrients for Various Population Groups of the Russian Federation (MR 2.3.1.0253-21). This document stipulates updated standards for recommended intake of various nutrients. The Standards rely on the latest research in the effect of bioactive substances on adaptative and protective mechanisms. They feature both macronutrients and micronutrients, including bioactive substances. For the first time, the Standards provide intake levels for phenolic compounds, e. g., phenolic acids, flavonoids, polymeric phenolic compounds, stilbenes, etc. As part of human diet, these substances reduce the risk of cardiovascular, oncological, and other diseases [3].

Phytoadaptogens are functional ingredients of food systems that facilitate antioxidative processes and encourage other protective substances to synthesize antistress proteins. Ginseng, ginger, chaga, garlic, and eleutherococcus are popular ingredients in functional drinks, bakery products, dairy spreads, food concentrates, etc. Plants owe their adaptogenic properties to such polyphenolic compounds as phenolic acids, stilbenes, flavonoids, lignans, etc. [4, 5]. Polyphenolics represent the largest group of natural antioxidants that protect cells from oxidative stress. In addition, they are anti-inflammatory, anti-carcinogenic, epigenetic, antimicrobial, metabolic, neuroprotective, and anti-age. If consumed in small quantities, they normalize metabolism [6, 7].

Physical fatigue in professional athletes reduces their performance. As a result, functional foods and additives are the necessary part of sports diet that facilitates recovery processes after heavy exercise. Plant polyphenols are reported to improve athletes' performance and inhibit fatigue processes induced by oxidative stress [8].

Many recent publications indicate the potential for finding effective plant-based adaptogens and developing new functional foods, additives, and medicines for various population groups.

Siberia and Russian Far East boast a unique diversity of wild flora that proliferates in harsh climate, which contributes to the accumulation of various bioactive substances. For example, *Pinus* trees are omnipresent and yield a remarkable range of raw materials. Scots pine (*Pinus sylvestris* L., 1753), Siberian pine (*Pinus sibirica* Du Tour), and Siberian dwarf pine (*Pinus pumila* (Pall.) Regel) are a source of needles, shoots, buds, bark, and cones that, as decoctions or infusions, have long been used to treat inflammatory diseases or as general tonics [9, 10]. Scots pine buds are a pharmacopoeial raw material: their decoctions are excellent expectorants. Cedar pine nuts are known to possess a rich and unique composition of nutrients and bioactive substances [11–13]. Regardless of the solvent, extraction method, species, and plant part, all pine extracts are extremely rich in polyphenols [14–16]. Pine materials have an enormous potential for functional foods and pharmacy [17–19], which remains untapped [20].

Pine nut oil cake is an abundant by-product of pine nut processing. It is a valuable source of plant proteins, essential amino acids, polyunsaturated fatty acids, vitamins, minerals, and dietary fiber, which makes it an ideal ingredient for functional foods [12, 21, 22].

Pine microstrobiles are male cones harvested in May and June before flowering. They are a valuable by-product of pine pollen. After ripening and de-pollenating, up to 90–95% of empty microstrobiles are discarded as waste. However, pine microstrobiles are rich in antioxidant phenolic compounds, as well as terpenoids that are famous for their antimicrobial and bactericidal properties [23, 24]. Therefore, along with pine nut cake, they are valuable raw ingredients for adaptogenic functional foods. In our previous research [25], we developed a herbal beverage with tea leaves, sea buckthorn cake, microstrobiles, and pine needles, as well as proved its adaptogenic effect.

Functional foods fortified with vitamins, minerals, dietary fiber, and phenolics are extremely popular. According to analytical data for 2020, the share of functional products of plant origin is 0.2%, i. e., about 11,000 tons. By 2029, this figure is projected to reach 10.7%, i. e., about 664,000 tons. Both the *Strategy for Improving Food Quality in the Russian Federation through 2030* and the *Doctrine of Food Security of the Russian Federation* see it that market growth is aimed at ensuring adequate nutrition, disease prevention, increasing life expectancy, and improving the quality of life, not only at production development [26]. In this regard, new formulations and technologies for adaptogenic functional foods are a national priority [27].

Busy lifestyles and schedules make consumers prefer healthy products that are easy to consume, store, and handle. Snack bars are consumer-friendly ready-to-eat products. They are made from grains, fruits, and nuts, which makes them a good source of nutrients, bioactive compounds, and dietary fiber [28–32].

In this research, we used secondary raw materials from Siberian dwarf pine to create a functional product and test its adaptogenic effect.

STUDY OBJECTS AND METHODS

The cake of peeled Siberian dwarf pine nuts (*Pinus pumila* (Pall.) Regel) was a by-product of oil pressing obtained at BaikalEkoProdukt LLC. The microstrobiles were waste from pollen harvesting. The nuts and pollen

were harvested in the Transbaikalian District of the Republic of Buryatia in 2023. The raw materials were dried indoors at 20–25°C away from direct sunlight.

The experimental functional snack bar also included taste additives. The dried sea buckthorn berries were harvested in the Selenga District of the Republic of Buryatia in 2023 while the sea buckthorn cake was a by-product of oil pressing. The flaxseed and date paste were purchased from retailers.

The pine nut cake was obtained by microwave heating to enhance the process of oil extraction with ethyl alcohol and pressing [33].

The dried sea buckthorn cake was obtained as in patent no. RU 2785625. Fresh or frozen sea buckthorn berries were dried in a vacuum microwave oven at 40–45°C. The pressure of 8.5–8.8 kPa resulted in 4–6% residual moisture. The dried berries then went through a microwave-powered hydraulic press at 40–45°C (8–10 min).

The flaxseed cake was obtained using the same method as the pine nut cake, i. e., in a microwave-powered hydraulic press [33].

We chose the dry mixing technology to make the snack bar as homogeneous as possible [34]. The pine nut cake, dried sea buckthorn, flaxseed, and pine microstrobiles were ground to a particle size of ≤ 1 mm and mixed. After adding the date paste, we mixed all components together for 6–8 min to form a layer that was 1.0–1.5 cm thick. Then, we cooled it down to 18 ± 3°C to be cut into bars of 50 g each (Patent no. RU 2834785 C1).

The nutritional value of the experimental snack bar included the content of proteins (State Standard GOST 10846-91), fats (State Standard GOST 29033-91), carbohydrates (State Standard GOST 26176-2019), and dietary fiber (State Standard GOST R 54014-2010).

The amino acid composition was determined by the method of high-performance liquid chromatography (HPLC) in an LA8080 automatic analyzer (Hitachi, Japan). The device included an 80×4.6 mm column, ion-exchange resin #2622, and a spectrophotometric detector. The pre- and post-column derivatizations suppressed the ammonia peak and modified amino acids with ninhydrin. The analyzer operated at 20–90°C column temperature; 125°C ninhydrin reactor temperature; 1.6 MPa operating pressure; 0.2 mL/min eluent flow rate; 440 nm (Channel 1); and 570 nm (Channel 2). The analysis followed an adapted program where five eluents with different compositions and pH were supplied at specific temperatures to facilitate the amino acid separation.

The method of gas chromatography-mass spectrometry made it possible to study **the fatty acid composition**. We modified the standard Bligh & Dyer method to extract lipids from the fat fraction [35]. The procedure involved an Agilent Packard HP 6890 N gas chromatograph (Germany) with a HP MSD 5973 quadrupole mass filter as a detector and a HP-5MS quartz column (30 m, 0.25 mm). Helium served as the carrier gas at a constant flow of 1.0–40 mL/min. The column temperatures were 125°C (0.5 min isotherm) and 125–320°C (7°C/min) while the evaporator temperature was 280°C. To define the percentage composition of fatty acids, we calculated the areas of gas chromatographic peaks. Then, we compared the retention times and full mass spectra of the corresponding pure compounds. The quantitative analysis relied on the NIST 11 data library and standard mixes of bacterial acid methyl esters (CP Mix, Supelco, Bellefonte, USA) and fatty acid methyl esters (Supelko 37 Component FAME Mix; 10 mg/mL in CH_2Cl_2).

To describe **the macro- and microelement composition**, we used the method of atomic absorption spectrophotometry on a Solaar M6 spectrometer (Thermo Scientific, USA). The preliminary digestion of samples with concentrated nitric acid involved a MARS 6 microwave system (CEM, USA) with XP-1500 Plus fluoropolymer-coated vessels. The standard reactor treatment procedure for plant samples with automatic temperature and pressure control was as follows: after the temperature was brought up to 200°C for 15 min, it remained 200°C at 2.96 MPa for another 15 min. All samples were analyzed in triplicate.

Vitamins C, B_2, B_3, B_6, and E, as well as carotenoids, were identified and measured in line with General Pharmacopoeia Monograph GPM.1.2.3.0017.15.

Total soluble polyphenols were determined using the Folin–Ciocalteu assay modified by Singleton *et al.* [36] with gallic acid for polyphenol calibration.

Total tannins were determined by the titrimetric method as described in General Pharmacopoeia Monograph GPM.1.5.3.0008.

To define **the antioxidant activity**, we used the total antioxidant content in the aqueous extracts (quercetin) and the lipid fraction (gallic acid). The amperometry followed the procedures in State Standard GOST 54037-2010 and involved a TsvetYauza-01-AA chromatograph (Russia).

The adaptogenic test involved 40 white mongrel mice (18–20 g) maintained in a standard vivarium with artificial lighting and free access to food and water (12:12 LD; 20–25°C; 60–70% relative humidity).

To model a cytostatic-induced immunosuppression, the mice were gavaged with a water solution of azathioprine for five days: 50 mg per 1 kg body weight per day. After gavaging, each mouse received 200 mg of the experimental snack bar (3% daily food intake) daily for 14 days. Table 1 illustrates the experimental design, with 10 mice per experimental group.

All animal experiments followed the humane principles and international ethical standards mentioned in

Table 1 Experiment design

Group	Dose
I (control)	Intact mice
II (azathioprine)	50 mg per 1 kg body weight, once a day, 5 days
III (azathioprine + experimental snack bar)	Azathioprine (50 mg per 1 kg body weight) + snack bar as feed additive (200 mg/mouse/day), 14 days
IV (snack bar)	Snack bar as feed additive (200 mg/mouse/day), 14 days

The European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (ETS 123) by the Council of Europe and the EU Directive 2010/63/EU. The care and handling relied on the procedures described in State Standard GOST 33216–2014 and Order 199-N of the Russian Ministry of Health.

The mouse behavior was filmed for 5 min as in Open Field Test [37]. A square white arena with an area of 1 m^2 and a 50 cm high side was mounted on a wooden frame. The field floor was made of Whatman paper divided into ten equal squares (10×10 cm). It was covered with glass to facilitate the visual recording of motor activity in the center (36 squares) and periphery (64 squares). An incandescent lamp (300 W) was located in the center at a height of 1 m above the arena floor.

The testing took place 1 h after the morning meal. The parameters to be registered involved: horizontal activity (peripheral and central squares crossed), vertical activity (rearing events with and without support on the side of the arena), grooming events, defecations, urinations, and atypical behavioral reactions. The total motor activity was the sum of horizontal and vertical activities. After testing each animal, the field was sanitized with a 70% ethyl alcohol solution and allowed to dry.

The integrated behavioral assessment followed the methodology developed by Buslovich *et al.* [38]. Exploratory activity consisted of latent time spent on leaving the center (–0.5 points for 1 s), horizontal activity (1 point for 1 sector), rearing events (3 points for 1 rearing without front-paw support and 2 points for 1 rearing with support), and entering the center (5 points). The overall anxiety level was the sum of points for short grooming events (1 point), boluses (1.5 points), urinations (2 points), and atypical behavior (4 points).

To assess **the overall physical activity**, we used the Porsolt forced swim test with a weight attached to the base of the tail (10% body weight) [39]. Each animal was placed in a vessel (diameter 18–20 cm, depth 40 cm) with pre-settled water (25–26°C). The mouse was allowed to swim until exhaustion, which started when the animal stayed underwater for ≥ 3 s. The effectiveness was assessed by comparing the swimming time in the experimental groups and the control group.

The mice were sacrificed to determine the weights of thymus, spleen, and liver, to collect blood, and perform a histomorphological analysis.

The relative organ weight was calculated as in Eq.:

$$X = \frac{Wa}{Wb} \times 100$$

where X is the relative organ weight (Me (Q1–Q3)); Wa is the absolute organ weight, g; and Wb is the body weight, g.

After weighing, the thymus and spleen were placed in 10% neutral buffered formalin. The cytomorphological examination followed a standard procedure. After processing, the organs were paraffined [40]. Then, histological sections of 5–6 μm thick were stained with Ehrlich's hematoxylin and eosin and embedded in Canada balsam. The morphometric analysis involved a Mikromed-3 U3 microscope equipped with a ToupCam 5.1 video eyepiece. Each experimental group had fifty microscoped sections.

The data obtained were processed in Microsoft Office Excel 2019 and presented as a median (Me) with upper and lower quartiles (Q_1–Q_3). Statistical significance was determined using the nonparametric Mann–Whitney test ($p \leq 0.05$).

RESULTS AND DISCUSSION

The formulation and macro- and micronutrient profile of the experimental snack bar. Fruit, berry, and grain snack bars are popular among adult and juvenile athletes and tourists who stick to a healthy diet, not to mention sugar-free brands favored by diabetic patients [34]. By-products of plant processing have an enormous biotechnological potential: when used in functional foods, it promotes the sustainable use of natural resources and reduces the environmental impact of the food industry [41].

In this research, we attempted to utilize secondary raw materials from Siberian dwarf pine, i. e., pine nut cake and microstrobiles, as a functional snack bar and assess its effectiveness. Functional ingredients with physiological effects are not enough for a successful commercial product. The snack bar was to have an attractive sensory profile, a convenient shape, and a long shelf-life. In addition, it was to address some environmental issues of complex plant material processing. As a result, we fortified the initial formulation with sea buckthorn, flaxseed, and date paste. Table 2 gives the formulations in descending order of component mass fraction.

Each ingredient was to perform a specific function. Pine nut cake was a by-product of oil extraction from peeled pine kernels of *Pinus pumila* (Pall.) Regel. Other pine species, e. g., *Pinus sibirica* Du Tour or *Pinus koraiensis* Siebold & Zucc. are a reliable alternative. The pine nut cake was selected for its easily digestible proteins and essential amino acids, macro- and microelements, vitamins B, and dietary fiber, as well as polyunsaturated fatty acids with vitamin F [13, 22]. Pine nut cake and meal maintain the original pine nut flavor and aroma, which means no synthetic flavorings are required.

Pine microstrobiles are male cones of *Pinus* plants. After pollen is extracted, the resulting empty microstrobiles are dumped as waste, and it can be 90–95% of the original raw material weight. Microstrobiles are a valuable source of phenolic compounds and essential oils.

Table 2 Composition of experimental pine nut snack bar

Component	Ratio, wt.%
Pine nut cake	40.0–50.0
Date paste	35.0–45.0
Sea buckthorn cake	8.0–14.0
Flaxseed cake	3.0–5.0
Pine microstrobiles	2.0–3.0

As a result, microstrobiles can fortify functional foods with antioxidant properties while essential oils with their bactericidal properties provide a long shelf-life and a pleasant pine aroma. As microstrobiles are a by-product, their use contributes to sustainable production [23, 24].

Sea buckthorn (*Hippophae rhamnoides* L.) is the only plant source of unsaturated fatty acids, including *ω*-7 fatty acids, as well as tocopherols and phenolic compounds, which have antioxidant and anti-inflammatory properties. Dried buckhorn or its cake containing 25–30% of residual oil can increase the nutritional and biological value of the bar under study and enhance its sensory attributes (Patent no. RU 2785625) [42, 43].

Flaxseed (*Linum usitatissimum* L.) is a functional plant ingredient containing such main groups of biologically active substances as *α*-linolenic acid (*ω*-3 essential polyunsaturated fatty acids), lignans, and dietary fiber. In our experimental snack bar, we used flaxseed cake obtained as a by-product of flaxseed oil pressing. It was to fortify the finished product with *ω*-3 fatty acids, lignans, dietary fiber, and protein [44, 45].

Dates (*Phoenix dactylifera* L.) are a valuable source of such nutrients as monosaccharides (mainly fructose and glucose), dietary fiber, unsaturated fatty acids, trace elements, and phenolic antioxidants. These substances provide dates with anti-inflammatory, antitumor, antihypertensive, antihypercholesterolemic, antimicrobial, and prebiotic properties. We used date paste in our experimental snack bar as a binding component, natural sweetener, and a source of macro-, micronutrients, and polyphenolic compounds. Date paste had the necessary plasticizing properties to render the product with a soft, pliable, and non-breakable consistency [46–48].

In this study, the formulation and technology involved microwave processing, which provided a disinfecting effect. To mix the dry ingredients with the date paste, we also employed microwave heating to improve the microbiological properties, quality, and shelf-life of the snack bar. Microwaving during mixing allowed the finished product to preserve the original properties, i. e., flavor, color, aroma, and biological value. As a result, the snack bar retained the natural sensory profile of the original raw materials and maintained its microbiological stability after 180 days of storage. The extended shelf-life was also due to the antioxidant-rich ingredients in the formulation.

We conducted seven series of tests to evaluate the composition and optimize the component ratio (Table 3).

When the component ratio was 35.0–38.0% of pine nut cake, 47.0–55.0% date paste, and 8.0–10.0% flaxseed cake (Variants 1 and 2), the snack bar was too soft and sweet, with not enough nut and berry flavor. When the date paste content was 25.0–30.0% (Variants 6 and 7), the snack bar was not plastic enough: the excess of dry components made it crumbly. The 10.0–14.0% range of sea buckthorn cake gave the experimental snack bar a pleasant berry flavor and aroma while 6.0% was not enough to improve the sensory properties and ≥ 14% spoiled the appearance. The optimal share of pine microstrobiles was 2.0–3.0%: a lower content failed to improve the sensory properties while a higher content gave the bar a bitter aftertaste of pine resin. Variants 3–5 demonstrated the best sensory scores: 40.0–45.0% of pine nut cake, 35.0–45.0% date paste, 8.0–14.0% dried sea buckthorn cake, 3.0–5.0% flaxseed cake, and 2.0–3.0% pine microstrobiles. These formulations provided optimal texture and plasticity with steady flavor and aroma (Patent no. RU 2834785 C1).

For our further experiments, we used Variant 4. Tables 4 and 5 illustrate the sensory properties of the experimental snack bar and its nutritional value.

Based on its chemical composition, the energy value of 100 g of the experimental snack bar was 304 kcal, i. e., 18.1% of the recommended daily intake for an adult aged 16–40 y. o. The following components exceeded 30% of the recommended daily intake for an adult (per 100 g of the experimental snack bar): protein (≥ 20.0% energy value of the snack bar), dietary fiber (6.2 g), essential amino acids (valine, isoleucine, methionine + cysteine, arginine), polyunsaturated fatty acids, including *ω*-3 and *ω*-6 (66.0–72.0%); potassium, phosphorus, magnesium, iron, manganese, zinc, copper, vitamin C,

Table 3 Component ratio across formulations, wt.%

Component	Variant 1	Variant 2	Variant 3	Variant 4	Variant 5	Variant 6	Variant 7
Pine nut cake	35.0	38.0	40.0	42.0	50.0	50.0	53.0
Date paste	55.0	47.0	45.0	38.0	35.0	30.0	25.0
Sea buckthorn cake	0.0	6.0	8.0	14.0	9.0	15.0	17.0
Flaxseed cake	10.0	8.0	5.0	4.0	3.0	1.0	0
Pine microstrobiles	0.0	1.0	2.0	3.0	3.0	4.0	5.0

Table 4 Sensory profile of pine nut snack bar

Indicator	Properties
Taste and aroma	Typical of raw ingredients, pleasant, slightly sweet, steady, with a nutty aftertaste
Surface	Smooth, slightly rough
Texture	Soft, viscous and plastic, homogeneous or with inclusions of pine nut cake flakes
Color	Homogeneous, uniform, orange-brown

Table 5 Nutritional profile of experimental snack bar (mean values)

Components	Content, 100 g/bar 50 g	Recommended daily intake (MR 2.3.1.0253-21)	% average recommended daily intake, per 100 g (per snack bar 50 g)
Calories, kcal	304/152	1,684	18.1 (9.1)
Proteins, g	20.4/10.2	75.0	26.8 (13.4)
Fats, g	10.0/5.0	83.0	17.8 (8.9)
Carbohydrates, g	28.8/14.4	365.0	13.2 (6.6)
Fiber, g	6.2/3.1	20.0	31.0 (15.5)
Amino acids, mg			
Valine	0.8/0.4	2.5	32.0 (16.0)
Isoleucine	0.6/0.3	2.0	30.0 (15.0)
Leucine	1.2/0.6	4.6	26.1 (13.1)
Lysin	0.6/0.3	4.1	14.6 (7.3)
Methionine + cystine	0.6/0.3	1.8	33.3 (16.7)
Threonine	0.6/0.3	2.4	25.0 (12.5)
Phenylalanine + tyrosine	1.2/0.6	4.4	27.3 (13.7)
Agrinine	3.0/1.5	6.1	49.2 (24.6)
Histidine	0.4/0.2	2.1	19.1 (9.6)
Alanine	0.8/0.4	6.6	12.1 (6.1)
Aspartic acid	1.5/0.8	12.2	12.3 (6.2)
Glycine	0.8/0.4	3.5	22.8 (11.4)
Glutamic acid	3.6/1.8	13.6	26.5 (13.3)
Proline	0.8/0.4	4.5	17.8 (8.9)
Serine	1.0/0.5	8.3	12.0 (6.0)
Fatty acids, mg			
Saturated fatty acids	1.5/0.8	25.0	6.1 (3.0)
Monounsaturated fatty acids	2.5/1.3	30.0	8.4 (4.2)
Polyunsaturated fatty acids	7.9/4.0	11.0	71.8 (35.9)
ω-3 fatty acids	0.66/0.33	1.0	66.0 (33.0)
ω-6 fatty acids	7.0/3.5	10.0	70.0 (35.0)
Macro- and microelements, mg*			
Potassium	958.4/479.2	3,500.0	38.3 (19.2)
Phosphorus	493.5/246.8	700.0	61.6 (30.8)
Calcium	14.2/7.6	1,000.0	1.4 (0.8)
Magnesium	192.8/96.4	420.0	48.2 (24.1)
Iron	4.2/2.1	10.0	42.0 (21.0)
Manganese**	7.8/3.9	2.0	390.0 (195.0)
Zink	5.6/2.8	12.0	46.7 (23.4)
Copper**	1.2/0.6	1.0	120.0 (60.0)
Vitamins, mg*			
C	38.2/19.1	100.0	38.2 (19.1)
B_2 (riboflavin)**	9.6/4.9	2.0	480.0 (245.0)
B_6 (pyridoxine)	2.1/1.1	2.0	105.0 (57.5)
B_3 (pantothenic acid)	7.0/3.5	5.0	140.0 (70.0)
Carotenoids, incl.	7.3/3.7	15.0	48.7 (24.4)
E	1.2/0.6	15.0	8.0 (4.0)

* The mean daily intake for vitamins and minerals is given for adults

** The content per 100 g is below the upper permissible daily intake level

vitamins B (riboflavin, pyridoxine, pantothenic acid), and carotenoids. In addition, 100 g of the experimental snack bar contained ≥ 15.0% of the average daily intake of essential amino acids (leucine, threonine, phenylalanine + tyrosine, histidine) and nonessential amino acids (glycine, glutamic acid, proline).

Table 6 shows the content of polyphenols and antioxidants in the experimental snack bar, which proved to be able to provide the daily intake of phenolic biologically active substances.

The resulting pine nut snack bar proved to be highly nutritional and functional. It could be classified as superfood because the amount of beneficial ingredients per unit of weight was maximal [49]. In addition, it was free of artificial preservatives, dyers, flavorings, and food additives.

Table 6 Total content polyphenols and antioxidants of pine nut bar

Indicator	Content, mg/100 g	Recommended daily intake (MR 2.3.1.0253-21), mg/24 h	% recommended daily intake
Total content of soluble polyphenols (Folin–Ciocalteu)	400.0	402	99.5
Total tannins expressed (tannin)	420.0	400	105.0
Total water-soluble antioxidants (quercetin)	503.0	322	156.0
Total fat-soluble antioxidants (gallic acid)	9.8	50	19.6

Table 7 Effect of experimental pine nut snack bar on mice behavior, Open Field, Me (Q_1–Q_3)

Group	Horizontal activity, events		Vertical activity, events	Grooming, events	Boluses	Urinations
	Peripheral squares	Central squares				
I (control)	146.4 (132.4–160.4)	6.4 (4.0–8.0)	3.3 (2.9–3.7)	1.2 (0.8–1.5)	1.7 (1.2–2.2)	1 per group
II (azathioprine)	90.8[a] (82.6–98.9)	1.0[a] (0.6–1.4)	5.9[a] (4.0–7.1)	1.7 (1.3–2.1)	1.9 (1.6–2.2)	2 per group
III (azathioprine + snack bar)	155.9[b] (141.4–170.5)	6.7[b] (5.2–7.9)	8.8[a,b] (7.2–10.3)	1.6 (1.2–2.0)	1.9 (1.3–2.5)	1 per group
IV (snack bar)	152.0 (133.1–170.9)	2.6[a] (1.8–3.4)	7.4[a] (6.4–8.5)	1.9 (1.4–2.4)	1.4 (0.9–1.9)	1 per group

[a, b] – the deviation is statistically significant in relation to groups I and II, respectively ($p \leq 0.05$)

Evaluating the adaptogenic properties of experimental snack bar. Adaptogens are natural or synthetic compounds that stimulate the immune system to resist biological, physical, chemical, emotional, and psychological stress.

We modified the Open Field Test to study the adaptogenic properties of the experimental snack bar. The test was designed to study the behavior of rodents under stress (Table 7, Fig. 1–3).

Azathioprine immunosuppression (Group II) decreased horizontal activity: the animals were by 38.0% and 84.4% less likely to cross the peripheral and central squares, respectively, but their vertical activity (rearings) increased by 78.8% ($p \leq 0.05$). The other indicators did not change significantly. The snack bar and azathioprine (Group III) raised the horizontal activity events to the control values. Their vertical activity also increased, compared to Groups I and II ($p \leq 0.05$). Group IV (snack bar, no azathioprine) had the same score for peripheral activity as the control group while the central activity was 2.5 times lower and the vertical activity was 2.2 times higher ($p \leq 0.05$). Other changes were insignificant ($p > 0.05$).

As for the overall physical activity (Fig. 1), the pattern was as follows: azathioprine reduced motor activity by 40% (Group II) while the subsequent introduction of the bar into the diet (Group III) brought it back to the control level ($p \leq 0.05$). When the bar was introduced into the diet of intact animals (Group IV), this indicator remained unaffected ($p > 0.05$).

Similar changes occurred in the exploratory activity (Fig. 2). As an immunosuppressant, azathioprine reduced this indicator by 37.4% (Group II), while adding the snack bar to the diet (Group III) restored it to the control level ($p \leq 0.05$). The intact animals (Group IV) showed no changes in the exploratory patterns ($p > 0.05$) after the snack bar became part of their diet.

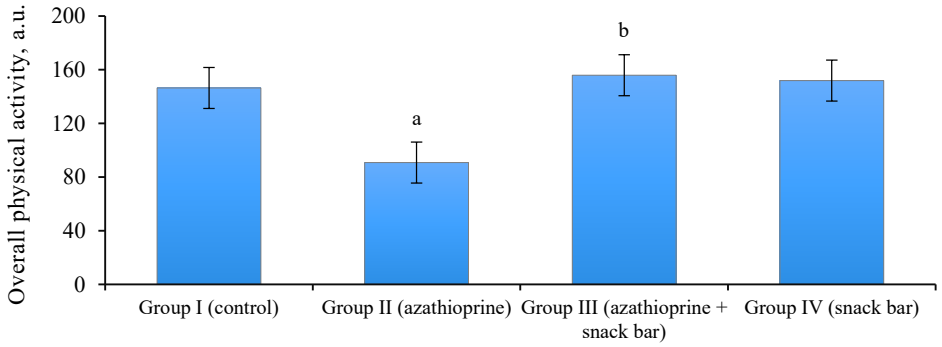
The integrated anxiety assessment (Fig. 3) showed that azathioprine increased anxiety by 14.9% while the combination of snack bar and azathioprine stabilized it ($p \leq 0.05$). The intact animals that ate the snack bar (Group IV) did not change their anxiety pattern ($p > 0.05$).

Figure 4 shows the physical performance of mice in the Porsolt Forced Swim Test with a weight load (10% of body weight). Swimming is a strenuous dynamic physical activity. Azathioprine (Group II) reduced the physical performance by 33.0%, and the combination of snack bar and azathioprine (Group III) brought this indicator back to the control values ($p \leq 0.05$). The intact animals (Group IV) showed no changes in the physical performance patterns ($p > 0.05$) after the snack bar became part of their diet.

The bar was rich in vitamins C and E, carotenoids, and polyphenols, as well as water- and fat-soluble antioxidants. Their antioxidant bioactive components exhibited pronounced adaptogenic effect on mice with azathioprine-induced immunosuppression (Tables 5 and 6). The combined effect of its ingredients increased their overall physical and exploratory activity, improved endurance, and decreased anxiety.

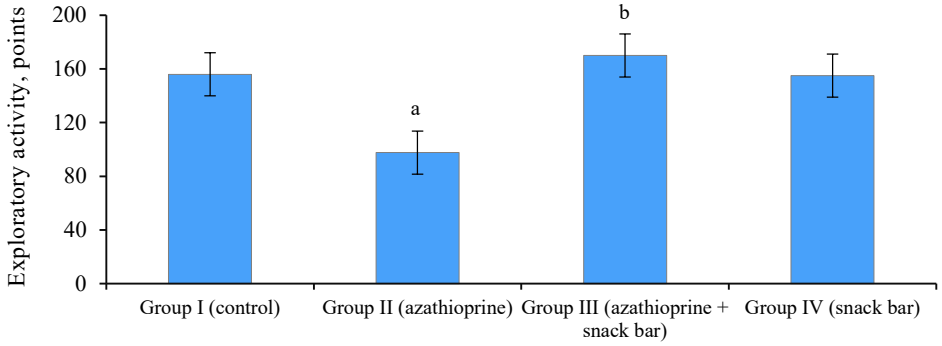
The azathioprine-induced immunosuppression made it possible to study the effect of the experimental snack bar on the relative weights of immune system organs, i. e., thymus and spleen, as well as liver as responsible for detoxification (Table 8).

The azathioprine-induced immunosuppression reduced the relative weights of thymus and spleen and increased the liver weight (Group II) ($p \leq 0.05$). The experimental snack bar in the diet (Group III) compensated



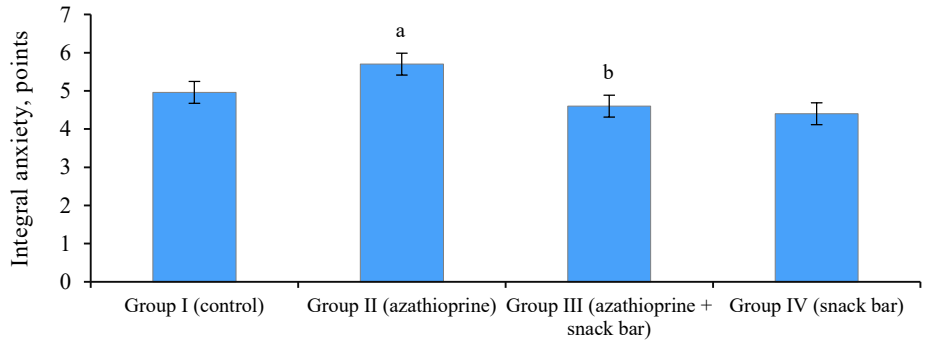
[a, b] – the deviation was statistically significant in relation to Groups I (control) and II (azathioprine), respectively ($p \leq 0.05$)

Figure 1 Overall physical activity in mice



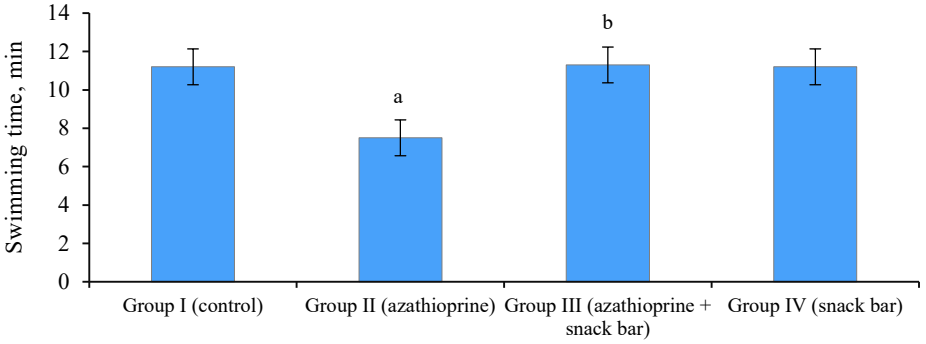
[a, b] – the deviation was statistically significant in relation to Groups I (control) and II (azathioprine), respectively ($p \leq 0.05$)

Figure 2 Exploratory activity in mice



[a, b] – the deviation was statistically significant in relation to Groups I (control) and II (azathioprine), respectively ($p \leq 0.05$)

Figure 3 Integral anxiety assessment in mice



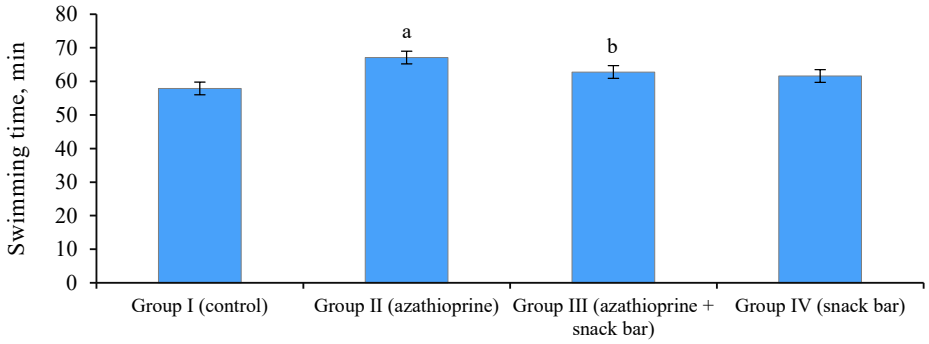
[a, b] – the deviation was statistically significant in relation to Groups I (control) and II (azathioprine), respectively ($p \leq 0.05$)

Figure 4 Physical performance in mice: Swimming time

Table 8 Effect of experimental pine nut snack bar on relative organ weight

Group	Relative weight, Me (Q_1–Q_3)		
	Thymus	Spleen	Liver
I (control)	0.15 (0.11–0.18)	0.45 (0.42–0.48	4.88 (4.06–5.53)
II (azathioprine)	0.09 (0.07–0.10)[a]	0.32 (0.31–0.33)[a]	5.74 (5.54–5.94)[a]
III (azathioprine + snack bar)	0.12 (0.09–0.15)	0.45 (0.40–0.50)[b]	4.78 (4.47–5.08)[b]
IV (snack bar)	0.14 (0.11–0.17)	0.46 (0.42–0.50)	4.41 (3.87–4.94)

[a, b] – the deviation was statistically significant in relation to Groups I (control) and II (azathioprine), respectively ($p \leq 0.05$)



[a, b] – the deviation was statistically significant in relation to Groups I (control) and II (azathioprine), respectively ($p \leq 0.05$)

Figure 5 Total antioxidant content in murine blood

for the negative effects of azathioprine and stabilized these parameters ($p \leq 0.05$). However, the snack bar had no effect on the relative organ weights of intact animals (Group IV).

We also identified the total antioxidant content in the murine blood (Fig. 5). Azathioprine (Group II) increased this parameter while the experimental snack bar (Group III) helped to stabilize it ($p \leq 0.05$). When added to the diet of the intact animals (Group IV), the snack bar had no effect on the total antioxidant content. The amperometry detected an increase in the total antioxidant content following the administration of azathioprine (Group II). This phenomenon may be due to the chemical structure of azathioprine and requires further research by different antioxidant assessment methods.

The azathioprine-induced immunosuppression inhibited the overall physical activity, exploratory patterns, and physical endurance while increasing anxiety. It reduced the relative weight of immune organs while increasing the relative weight of the liver, as well as led to high total antioxidant content in the blood (Tables 7 and 8, Fig. 1–5). When we introduced the bar into the diet during the azathioprine-induced immunosuppression, it reduced the negative impact of azathioprine and restored the abovementioned parameters to the control values ($p \leq 0.05$).

Histomorphology of thymus and spleen. The final stage of our research involved histomorphological studies of the murine immune system organs, i. e., thymus and spleen.

The thymus preparations (Table 9) obtained from the intact mice consisted of two asymmetrical lobes (Group I, Fig. 6). Each individual lobe was externally covered by

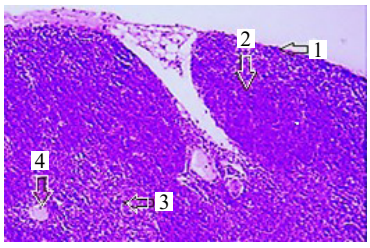


Figure 6 Histosected thymus in Group I (control): 1 – capsule; 2 – cortex; 3 – medulla; and 4 – Hassall's corpuscle (hematoxylin and eosin staining, PL 10×/0.25)

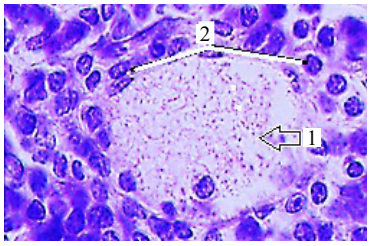


Figure 7 Nucleated thymus cells in Group I (control): 1 – Hassall's corpuscle; 2 – nucleated cells (hematoxylin and eosin staining, PL 10×/0.25)

a connective tissue capsule, from which thin septa (trabeculae) extended. Each thymus lobe had cortical and medulla zones with an obscure boundary between them. The cortical zone was 21.0–53.0 µm thick; the medulla zone was 53.0–100.0 µm long and 25.0–100 µm wide. The Hassall's corpuscles in the medulla zone consisted of keratinized epithelial cells (Fig. 7). Their count varied from 5 to 8 while the size ranged from 5 to 7 µm.

Table 9 Structure sizes and nucleated cell counts in thymus, Me (Q_1–Q_3)

Group	Thymus					
	Capsule, thickness, μm	Thymic corpuscle, diameter, μm	Cortex, thickness, μm	Medulla, length, μm	Medulla, width, μm	Nucleated cell count per section, cells
I (control)	4.8 (3.3–6.3)	6.5 (5.5–7.0)	35.5 (21.5–49.5)	78.0 (58.0–93.0)	40.0 (23.5–60.0)	52.0 (45.0–60.0)
II (azathioprine)	6.5 (5.0–8.0)	5.3 (3.7–7.0)	35.0 (20.0–50.0)	95.0[a] (94.0–105.0)	45.0 (35.5–57.0)	38.0[a] (32.0–44.0)
III (azathioprine + snack bar)	4.5 (4.0-5.5)	22.0[a, b] (17.0–27.0)	25.0 (21.0–29.0)	85.0[b] (75.0–93.0)	85.0[b] (59.0–113.0)	50.0[b] (45.0–55.0)
IV (snack bar)	5.5 (5.0-6.5)	6.5 (4.0–7.0)	25.0 (15.0–43.0)	95.0 (65.0–125.0)	45.0 (25.0–65.0)	115.0[a] (110.0–119.0)

[a, b] – the deviation was statistically significant in relation to Groups I (control) and II (azathioprine), respectively ($p \leq 0.05$)

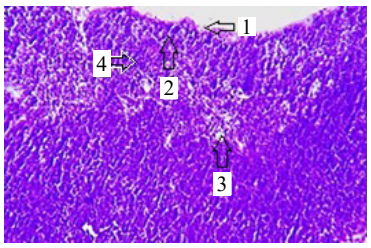


Figure 8 Histosected thymus in Group II (azathioprine): 1 – capsule; 2 – medulla; 3 – cortex; and 4 – inverted layers (hematoxylin and eosin staining, PL 10×/0.25)

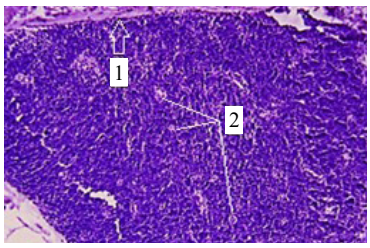


Figure 9 Histosected thymus in Group III (azathioprine + snack bar): 1 – capsule; 2 – enlarged Hassall's corpuscles (hematoxylin and eosin staining, PL 10×/0.25)

The medulla-to-cortex ratio in the intact mice ranged from 1:1.1 to 1:2. The count of nucleated thymus cells in sections (Fig. 7) averaged 52.0 ± 7.0 (Table 9).

Oral administration of azathioprine to Group II changed the structural microanatomy of the thymus tissue. The boundaries between the cortex and medulla were blurred in most preparations. The cortex was visible as a light zone while the medulla demonstrated some dark zones (layer inversion) (Fig. 8). Histological studies in Group II revealed some thickening and loosening of the capsule. The thymus lobule had fewer (4–6) and smaller (3–8 μm) thymic bodies than in the intact mice (Group 1). Some areas of the lobules accumulated fat cells on the capsule surface. The fibroblastic ridges in the subcapsular zone of most preparations resulted from the azathioprine-induced disturbances in the lymphoid tissue. Morphometrically, the length and width of the medulla increased by 21.8 and 12.5%, respectively, while the nucleated thymic cell count decreased by 26.9% compared to the control group ($p \leq 0.05$) (Table 9).

Group III that received the experimental bar after the administration of azathioprine demonstrated structural changes in the thymus. The border between the cortex and medulla was better visible. Compared with Group II, they had a 1.1 times shorter and 1.9 times wider medulla ($p \leq 0.05$). The capsule was 1.4 times thinner, but its parameters gradually returned to the control level. Group III had more thymic Hassall's corpuscles, and they were bigger (Fig. 9). Their count increased from 6 to 9 while the diameter grew by 4.2 times ($p \leq 0.05$), compared with Group II. The diameter was also reliably higher

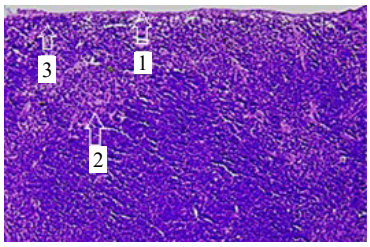


Figure 10 Histosected thymus in Group IV (snack bar): 1 – capsule; 2 – medulla; and 3 – cortex (hematoxylin and eosin staining, PL 10×/0.25)

(by 3.4 times) than in the control group. The nucleated cell count returned to the control level ($p \leq 0.05$). The observed changes could be recognized as a microanatomic restoration of the thymus structure.

After eating the experimental snack bar, the intact animals (Group IV) developed minor changes in the thymus microanatomy, compared to the control group. Most sections showed some loosening and slight thickening of the capsule ($p > 0.05$) (Fig. 10). Some histological sections of the thymus showed blurred boundaries between the lobes, cortex, and medulla. The cortex became thinner while the medulla became longer and wider, but these changes were insignificant ($p > 0.05$). Group IV also demonstrated a decrease in the count and diameter of thymic bodies (from 4 to 5) while the nucleated cell count increased by a factor of 2.2 ($p \leq 0.05$), compared to the control group.

Table 10 Morphometry of white pulp in murine spleen, Me (Q_1–Q_3)

Group	Mice spleen structure			
	White pulp mean area, μm²	Periarterial lymphoid sheath, thickness, μm	Lymphatic nodules, diameter, μm	Germinal center, diameter, μm
I (control)	220.0 (185.0–255.0)	4.6 (3.5–6.0)	20.0 (15.0–19.5)	6.2 (4.8–8.5)
II (azathioprine)	108.0[a] (68.0–148.0)	3.0[a] (2.2–3.4)	15.0 (13.5–17.0)	4.0[a] (2.5–4.5)
III (azathioprine + snack bar)	160.0 (110.0–210.0)	4.2 (2.7–5.7)	22.0[b] (19.5–24.5)	5.5 (3.0–8.0)
IV (snack bar)	225.0 (140.0–310.0)	4.5 (3.5–5.5)	22.5 (10.5–29.5)	6.0 (4.5–7.5)

[a, b] – the deviation was statistically significant in relation to Groups I (control) and II (azathioprine), respectively ($p \leq 0.05$)

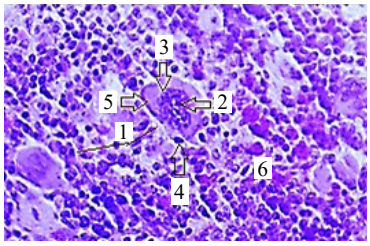


Figure 11 Histosected spleen in Group I (control): 1 – lymphoid follicle; 2 – germinal center; 3 – mantle zone; 4 – periarterial lymphoid cuff; 5 – marginal zone; and 6 – red pulp (hematoxylin and eosin staining, PL 40×/0.65)

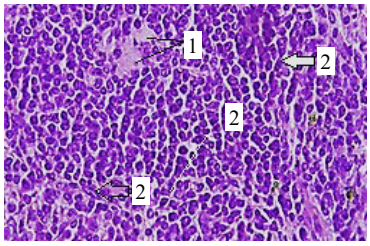


Figure 12 Histosected spleen in Group II (azathioprine): 1 – suppressed germinal centers, hyperplasia; 2 – nodules with irregular shapes (hematoxylin and eosin staining, PL 40×/0.65)

The spleen is another major organ of the immune system. In control Group I, spleen histology demonstrated primary nodules without germinal centers and secondary nodules with germinal centers. The spleen preparations revealed parenchyma, formed by white and red pulp. The histomorphological studies revealed a 2:1 ratio of secondary to primary lymphoid nodules per field of view. The white pulp consisted of equal asymmetrical lymphatic nodules (follicles) with a diameter ranging from 10.0 to 19.5 μm. The average area of lymphoid follicles in the white pulp was 220.0 μm² (Table 10). A darker-colored germinal core (6.2 μm thick) was observed in the center of the lymph node around the central arteries. The periarterial lymphoid sheath diameter in Group I ranged from 3.5 to 6.0 μm (Table 10, Fig. 11). Some marginal and mantle zones were visible in the periphery of the lymphoid follicles.

Table 10 summarizes the structural morphometry of the murine spleen samples.

Administration of azathioprine in Group II changed the structure of the spleen (Fig. 12). Most histological preparations showed large irregular lymphoid follicles with uneven thickness, as well as a 1.3-fold decrease in follicle area, compared to the control group (Table 10). In Group II, the significant decrease in follicle area was accompanied by a 1.5-time decrease in the thickness of periarterial lymphoid sheath while the size of germinal centers decreased by a factor of 1.6, compared to the intact mice ($p \leq 0.05$). The ratio between secondary and primary lymphoid nodules in spleen preparations

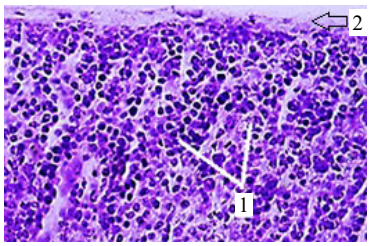


Figure 13 Histosected spleen in Group III (azathioprine + snack bar): 1 – enlarged lymphoid nodules; 2 – capsule (hematoxylin and eosin staining, PL 40×/0.65)

was 1:1 per field of view. Azathioprine caused a sharp decrease in spleen activity with advanced white pulp hyperplasia (Fig. 12).

The mice that received the snack bar during immunosuppression (Group III) showed significant changes in spleen structure compared to Group II. The histology demonstrated more lymph nodes with germinal centers (Fig. 13). Other increased parameters included the average area of the white pulp (1.5 times), the thickness of the periarterial lymphoid sheath (1.4 times), the diameter of the lymphoid nodes (1.5 times) ($p \leq 0.05$), and the diameter of the germinal centers (1.4 times) (Table 10). Apparently, the parameters were restored to the control level (Group I).

The intact mice that received the snack bar with feed (Group IV) demonstrated no significant changes in such spleen parameters as the average white pulp area, the

thickness of the periarterial lymphoid sheath, the diameter of lymphoid nodules, and the diameter of germinal centers ($p > 0.05$). (Table 10, Fig. 14).

The oral administration of azathioprine triggered significant morphological changes in the murine thymus and spleen, indicating their poorer functional activity. The count and size of thymic Hassall's corpuscles went down, and so did the nucleated cell count. The spleen demonstrated a reliable decrease in the average area of follicles and the thickness of germinal centers, compared to those in the intact group. Other publications also reported similar azathioprine-induced damage to the spleen and involutional changes in the thymus [50, 51]. The experimental snack bar introduced against the background of azathioprine immunodepression increased the cortex-to-medulla ratio in the thymus. The histology showed a reliable increase in the thickness of the follicles (1.5 times), compared to those in Group II. The structural parameters of the thymus and spleen in Group III approximated the values in the control group. Group IV that received the snack bar but no azathioprine demonstrated minor changes in the structural parameters of the immune organs ($p > 0.05$). The bar exerted its adaptogenic effect primarily against the background of the cytostatic azathioprine, i. e., under extreme exposure, which met the formal requirements for adaptogens.

The chemical properties and biological effects of plant metabolites and polyphenols are a popular research topic. Lyubitelev *et al.* [52] described the structure and properties of plant polyphenols, as well as their effect on oxidative stress. They reported high antioxidant activity: plant polyphenols both neutralize the existing free radicals and prevent their formation. They target Nrf2/Keap1 proteins that are involved in the cellular redox metabolism. Tavan *et al.* [53] reviewed major publications on phenolic acids, flavonoids, stilbenes, and coumarins; they remarked upon their therapeutic potential and mechanisms of action in animal models. Quesada-Vázquez *et al.* [54] summarized the effect of polyphenols in preventing cancer, cardiovascular diseases, neurological disorders, liver diseases, etc. They reported some prospective topics, as well as potential problems and risks that researchers of phenolic compounds may face in the coming years:

– toxicology and safety risk assessment;
– synergistic effects between different polyphenols;
– diet recommendations based on the differential metabolism of polyphenols by intestinal microflora;
– consortia between polyphenols and other bioactive compounds;
– formulations that improve the bioavailability of phenolic compounds; as well as
– polyphenols in sports nutrition and recovery diets.

By-products of plant processing yield such valuable biological substances as polyphenols, carotenoids, terpenoids, alkaloids, saponins, vitamins, etc. Due to their wide range of biological activities, they can be used in the food industry, pharmaceuticals, and cosmetology [55].

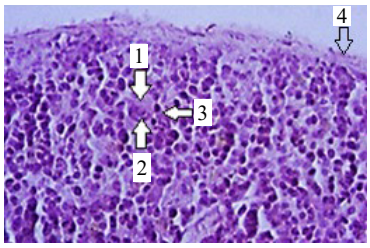


Figure 14 Histosected spleen in Group IV (snack bar): 1 – lymphoid nodule; 2 – germinal center; 3 – periarterial lymphoid sheath; and 4 – capsule (hematoxylin and eosin staining, PL 40×/0.65)

Consumers favor natural ingredients, herbal pharmaceuticals, and folk medicine, which gives polyphenols certain commercial advantages in maintaining human health. Not only do they promote environmental awareness but they also are associated with a zero-waste sustainable production cycle since peels, pomace, cakes, and other by-products are rich in polyphenols [56].

Russian scientists develop new formulations and technologies of complex adaptogenic food supplements that target various population groups [57]. For instance, food modelling technologies and algorithms rely on advanced food combinatorics and digital profiling [58].

Adaptogens are food agents that help people to adapt to such adverse factors as physical and mental stress. In this respect, polyphenols are the most advanced bioactive substances of plant origin. Their biochemical potential offers both scientific and applied use. Foods fortified with phytoadaptogens expand the range of functional products that facilitate consumers' adaptability to a changing environment by improving their resistance to various stresses.

CONCLUSION

This experimental research allowed us to develop a new functional snack bar from pine nut cake and pine microstrobiles with dried sea buckthorn, flaxseed cake, and date paste. It proved to have an advantageous chemical composition and bioactive profile: 100 g of snack bar contained ≥ 30% average daily intake of such macro- and micronutrients as dietary fiber, essential amino acids, polyunsaturated fatty acids, vitamins, minerals, and minor bioactive substances. In addition, it proved able to satisfy the daily requirement for bioactive polyphenols.

The experimental snack bar demonstrated reliable adaptogenic properties. When introduced into the diet of mice with azathioprine-induced immunosuppression, it helped to restore the structure and function of immune system organs, i. e., thymus and spleen. The recovery processes were modulating: the mice gradually restored their initial overall physical activity, exploratory patterns, and endurance, as well as returned to normal anxiety levels. The recovery of nonspecific resistance during immunosuppression was apparently related to the antioxidant properties of the functional

pine nut snack bar. The experiment on mice proved that it could be recommended as a dietary supplement for consumers of all ages that are subjected to environmental pollution or physical exertion, as well as to patients during recovery.

Plant-based by-products possess strong biotechnological potential for the functional food industry while contributing to sustainable production and reducing environmental impact.

CONTRIBUTION

The authors contributed equally to the manuscript and bear equal responsibility for any plagiarism. S.D. Zhamsaranova and V.G. Shiretorova developed the research concept; V.G. Shiretorova and S.A. Erdyneeva were responsible for sampling, sample preparation, and chemical analysis; V.G. Shiretorova and T.I. Kotova performed the research; S.N. Lebedeva and A.A. Tykheev conducted the biological study; V.G. Shiretorova and S.N. Lebedeva drafted the manuscript; S.D. Zhamsaranova, V.G. Shiretorova, and A.G. Khanturgaev proofread the manuscript; S.D. Zhamsaranova and A.G. Khanturgaev supervised the research. All the authors were involved in editing, reviewing, and proofreading the manuscript. All the authors read and approved of the final version.

CONFLICT OF INTEREST

The authors reported no conflict of interest regarding the publication of this article.

REFERENCES

1. Arapova OI. Adaptation phenomenon. Theoretical review. Psychology. Psychophysiology. 2023;16(3):111–122. (In Russ.) https://elibrary.ru/EYPQSA
2. Lavin KM, Coen PM, Baptista LC, Bell MB, Drummer D, *et al*. State of knowledge on molecular adaptations to exercise in humans: Historical perspectives and future directions. Comprehensive Physiology. 2022;12(2):3193–3279. https://doi.org/10.1002/j.2040-4603.2022.tb00211.x
3. Devyatov AA, Fedorova TN, Berezhnoy DS, Stvolinskii SL, Tutelyan VA. Mechanisms of neuroprotective action of hesperetin and carnosine in focal ischemia of the brain in rats. Bulletin of Experimental Biology and Medicine. 2020;169:242–245. https://doi.org/10.1007/s10517-020-04859-w
4. Ryabokoneva LA, Sergeeva IYU, Anshukov AV, Permyakova LV. Phytoadaptogens as Functional Ingredients for Food Systems (Review). Agro-industrial complex of Russia. 2024;31(1):105–118. (In Russ.) https://doi.org/10.55934/2587-8824-2024-31-1-105-118
5. Sokurenko MS, Solovieva NL, Bessonov VV, Mazo VK. Polyphenolic compounds of the stilbenoid class: Classification, representatives, content in plant raw materials, structural features, use in the food industry and pharmacy. Problems of Nutrition. 2019;88(1):17–25. (In Russ.) https://doi.org/10.24411/0042-8833-2019-10002
6. Mathesius U. Flavonoid functions in plants and their interactions with other organisms. Plants. 2018;7(2):30. https://doi.org/10.3390/plants7020030
7. Bobrysheva TN, Anisimov GS, Zolotoreva MS, Bobryshev DV, Budkevich RO, *et al*. Polyphenols as promising biologically active compounds. Problems of Nutrition. 2023;92(1):92–107. (In Russ.) https://doi.org/10.33029/0042-8833-2023-92-1-92-107
8. Kornyakova VV, Badtieva VA, Balandin MYU. Exploiting dietary supplements with antioxidant properties for enhancing physical efficiency at the state of physical fatigue in sports. Problems of Nutrition. 2020;89(3):86–96. (In Russ.) https://elibrary.ru/OTZQZK
9. Nikolic MV, Jakovljevic VL, Bradic JV, Tomovic MT, Petrovic BP, *et al*. Korean and Siberian pine: Review of chemical composition and pharmacological profile. Acta Poloniae Pharmaceutica. 2022;79(6):785–797. https://doi.org/10.32383/APPDR/161040
10. Vasilyeva AG, Chirikova NK. Phenolic compounds of the needles of *Pinus Pumila* (Pall.) Regel growing in Yakutia. Arctic and Subarctic Natural Resources. 2021;26(3):136–143. (In Russ.) https://doi.org/10.31242/2618-9712-2021-26-3-136-143
11. Rogachev AD, Salakhutdinov NF. Chemical composition of *Pinus sibirica* (Pinaceae). Chemistry & Biodiversity. 2015;12(1):1–53. https://doi.org/10.1002/cbdv.201300195
12. Babich OO, Milent'eva IS, Ivanova SA, Pavsky VA, Kashirskikh EV, *et al*. The potential of pine nut as a component of sport nutrition. Foods and Raw Materials. 2017;5(2):170–177. https://doi.org/10.21603/2308-4057-2017-2-170-177
13. Giro TM, Moiseeva NE, Mokretsov IV, Sukhov MA. designing a recipe for dry-cured sausages from the meat of fished animals. Bulletin of the South Ural State University Series Food and Biotechnology. 2025;13(2):52–63. (In Russ.) https://doi.org/10.14529/food250206
14. Shpatov AV, Popov SA, Salnikova OI, Kukina TP, Shmidt EN, *et al*. Composition and bioactivity of lipophilic metabolites from needles and twigs of Korean and Siberian pines (*Pinus koraiensis* Siebold & Zucc. and *Pinus sibirica* Du Tour). Chemistry and Biodiversity. 2017;14(2):e1600203. https://doi.org/10.1002/cbdv.201600203

15. Li H, Wang Z, Xu Y, Sun G. Pine polyphenols from *Pinus koraiensis* prevent injuries induced by gamma radiation in mice. PeerJ. 2016;4:e1870. https://doi.org/10.7717/peerj.1870

16. Huang Y, Zhu X, Zhu Y, Wang Z. *Pinus koraiensis* polyphenols: Structural identification, *in vitro* antioxidant activity, immune function and inhibition of cancer cell proliferation. Food & Function. 2021;12(9):4176–4198. https://doi.org/10.1039/d0fo03347b

17. Takala R, Ramji DP, Choy E. The beneficial effects of pine nuts and its major fatty acid, pinolenic acid, on inflammation and metabolic perturbations in inflammatory disorders. International Journal of Molecular Sciences. 2023;24(2):1171. https://doi.org/10.3390/ijms24021171

18. Ramos PAB, Pereira C, Gomes AP, Neto RT, Almeida A, *et al*. Chemical characterisation, antioxidant and antibacterial activities of *Pinus pinaster* Ait. and *Pinus pinea* L. bark polar extracts: Prospecting forestry by-products as renewable sources of bioactive compounds. Applied Sciences. 2022;12(2):784. https://doi.org/10.3390/app12020784

19. Peng X, Feng C, Wang X, Gu H, Li J, *et al*. Chemical composition and antioxidant activity of essential oils from barks of *Pinus pumila* using microwave-assisted hydrodistillation after screw extrusion treatment. Industrial Crops and Products. 2021;166:113489. https://doi.org/10.1016/j.indcrop.2021.113489

20. Dziedziński M, Kobus-Cisowska J, Stachowiak B. *Pinus* species as prospective reserves of bioactive compounds with potential use in functional food–current state of knowledge. Plants. 2021;10(7):1306. https://doi.org/10.3390/plants10071306

21. Prosekov AYu, Dyshlyuk LS, Milent'eva IS, Pavsky VA, Ivanova SI, *et al*. Study of the biofunctional properties of cedar pine oil with the use of *in vitro* testing cultures. Foods and Raw Materials. 2018;6(1):136–143. https://doi.org/10.21603/2308-4057-2018-1-136-143

22. Khanturgaeva VA, Hamaganova IV. The analysis and research of the composition a protein-vitamins product from plant materials. Proceedings of the Voronezh State University of Engineering Technologies. 2022;84(1):49–57. (In Russ.) https://doi.org/10.20914/2310-1202-2022-1-49-57

23. Erdyneeva SA, Shiretorova VG, Radnaeva LD. Pharmacognostic study of *Pinus sylvestris* L. microstrobils. Problems of biological, medical and pharmaceutical chemistry. 2022;25(9):34−39. https://doi.org/10.29296/25877313-2022-09-05

24. Shiretorova VG, Erdyneeva SA, Radnaeva LD. Elemental composition of microstrobili and sprouts of *Pinus sylvestris*, *Pinus sibirica* and *Pinus pumila*. Proceedings of Universities. Applied Chemistry and Biotechnology. 2022;12(4):605–611. (In Russ.) https://doi.org/10.21285/2227-2925-2022-12-4-605-611

25. Zhamsaranova SD, Shiretorova VG, Erdyneeva SA, Tykheev AA, Lebedeva SN. Assessment of the adaptogenic properties of herbal tea based on pine needles and microstrobiles. Food Systems. 2025;8(1):124–133. https://doi.org/10.21323/2618-9771-2025-8-1-124-133

26. Asyakina LK, Loseva AI, Milent′eva IS, Prosekov AYu, Minina VI. Prospects for using Siberian federal district plants in the production of functional food products. Bulletin of the South Ural State University. Series. Food and Biotechnology. 2022;10(4):5–17. (In Russ.)

27. Piskunenko KR, Popov VG. Development of formulation and production technology for polyfunctional food ingredient with adaptogenic properties. Polzunovskiy vestnik. 2021;(3):141–146. (In Russ.) https://elibrary.ru/MVQIQH

28. Kowalska H, Kowalska J, Ignaczak A, Masiarz E, Domian E, *et al*. Development of a high-fibre multigrain bar technology with the addition of curly kale. Molecules. 2021;26(13):3939. https://doi.org/10.3390/molecules26133939

29. Haş IM, Vodnar DC, Bungau AF, Tarce AG, Tit DM, *et al*. Enhanced elderberry snack bars: A sensory, nutritional, and rheological evaluation. Foods. 2023;12(19):3544. https://doi.org/10.3390/foods12193544

30. Bratosin BC, Martău GA, Ciont C, Ranga F, Simon E, *et al*. Nutritional and physico-chemical characteristics of innovative bars enriched with *Aronia melanocarpa* by-product powder. Applied Sciences. 2024;14(6):2338. https://doi.org/10.3390/app14062338

31. AlJaloudi R, Al-Dabbas MM, Hamad HJ, Amara RA, Al-Bashabsheh Z, *et al*. Development and characterization of high-energy protein bars with enhanced antioxidant, chemical, nutritional, physical, and sensory properties. Foods. 2024;13(2):259. https://doi.org/10.3390/foods13020259

32. Cavalcanti ICA, Borges LA, de Carvalho MD, Pereira GSL, Oliveira MLP, *et al*. Development and evaluation of nutrient-rich food bars incorporating macauba kernel cake. Journal of Food Science and Technology. 2025;62:2191–2198. https://doi.org/10.1007/s13197-024-06166-y

33. Khanturgaev AG, Shiretorova VG, Kotova TI, Khanturgaeva VA. Production of functional products from Siberian pine seeds: Scientific basis and practical implementation: monograph. Ulan-Ude: ESSUTM; 2018, 140 p. (In Russ.)

34. Bakumenko OE, Ruban NV, Alekseenko EV. Scientific and practical aspects of developing snack bars for healthy nutrition. Kursk: University Book; 2023, 190 p. (In Russ.) https://doi.org/10.47581/2023/Bakumenko-Ruban.01

35. Breil C, Vian MA, Zemb T, Kunz W, Chemat F. "Bligh and Dyer" and Folch methods for solid–liquid–liquid extraction of lipids from microorganisms. Comprehension of Solvatation mechanisms and towards substitution with alternative solvents. International Journal of Molecular Sciences. 2017;18(4):708. https://doi.org/10.3390/ijms18040708
36. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in enzymology. 1999;299:152–178. https://doi.org/10.1016/S0076-6879(99)99017-1
37. Belyakov VI, Gromova DS, Popova NR, Myakisheva YuV. Modern methods for studying rodent behavior in model biomedical studies (problem review). Modern issues of Biomedicine. 2022;6(4):13–22. (In Russ.) https://doi.org/10.51871/2588-0500_2022_06_04_1
38. Buslovich SYu, Kotelenets AI, Fridlyand RM. Integral method for assessing the behavior of white rats in an open field. Journal of Higher Nervous Activity. 1989;39(1):168–170. (In Russ.)
39. Porsolt RD, Pichon ML, Jalfe M. Depression: A new animal model sensitive to antidepressant treatment. Nature. 1977;266:730–732. https://doi.org/10.1038/266730a0
40. Korzhevskiy DE, Gilyarov AV. Fundamentals of histological technique. St. Petersburg: SpetsLit; 2010, 95 p. (In Russ.)
41. Rațu RN, Veleșcu ID, Stoica F, Usturoi A, Arsenoaia VN, *et al*. Application of agri-food by-products in the food industry. Agriculture. 2023;13(8):1559. https://doi.org/10.3390/agriculture13081559
42. Chen Y, Cai Y, Wang K, Wang Y. Bioactive compounds in sea buckthorn and their efficacy in preventing and treating metabolic syndrome. Foods. 2023;12(10):1985. https://doi.org/10.3390/foods12101985
43. Wang Z, Zhao F, Wei P, Chai X, Hou G, *et al*. Phytochemistry, health benefits, and food applications of sea buckthorn (*Hippophae rhamnoides* L.): A comprehensive review. Frontiers in Nutrition. 2022;(9):1036295. https://doi.org/10.3389/fnut.2022.1036295
44. Noreen S, Tufail T, Ul Ain HB, Awuchi CG. Pharmacological, nutraceutical, and nutritional properties of flaxseed (*Linum usitatissimum*): An insight into its functionality and disease mitigation. Food Science & Nutrition. 2023;11(11):6820–6829. https://doi.org/10.1002/fsn3.3662
45. Niharika M, Sireesha G, Madhavi D, Sumalatha SV. Development of super food nutri rich bar. Journal of Health Sciences. 2021;8(4):254–259. https://doi.org/10.21276/apjhs.2021.8.4.25
46. Ayad AA, Williams LL, Gad El-Rab DA, Ayivi R, Colleran HL, *at al*. A review of the chemical composition, nutritional and health benefits of dates for their potential use in energy nutrition bars for athletes. Cogent Food & Agriculture. 2020;6(1):1809309. https://doi.org/10.1080/23311932.2020.1809309
47. Al-Karmadi A, Okoh AI. An overview of date (*Phoenix dactylifera*) fruits as an important global food resource. Foods. 2024;13(7):1024. https://doi.org/10.3390/foods13071024
48. Alfheeaid HA, Barakat H, Althwab SA, Musa KH, Malkova D. Nutritional and physicochemical characteristics of innovative high energy and protein fruit- and date-based bars. Foods. 2023;12(14):2777. https://doi.org/10.3390/foods12142777
49. Fernández-Ríos A, Laso J, Hoehn D, Amo-Setién FJ, Abajas-Bustillo R, *et al*. A critical review of superfoods from a holistic nutritional and environmental approach. Journal of Cleaner Production. 2022;379(Part 1):134491. https://doi.org/10.1016/j.jclepro.2022.134491
50. Tsyrenova DZ, Gulyaev SM, Khobrakova VB. Effect of *Phlomoides tuberosa* (L.) Moench extract on spleen structure in immunosuppressed mice. Reviews in Clinical Pharmacology and Drug Therapy. 2017;15(1):53–57. (In Russ.) https://doi.org/10.17816/RCF15153-57
51. Khobrakova VB, Razuvaeva YG, Budaeva ER. Correction of structural changes in thymus by Gentiana algida pall extract at experimental azathioprine immunosuppression. Antibiotics and Chemotherapy. 2020;65(7–8):18–22. (In Russ.) https://doi.org/10.37489/0235-2990-2020-65-7-8-18-22
52. Lyubitelev AV, Sivkina AL, Vlasova OA, Belitsky GA, Studitsky VM. Mechanisms of action of plant polyphenols on the initiation of carcinogenesis. Advances in Molecular Oncology. 2023;10(2):30–41. (In Russ.) https://doi.org/10.17650/2313-805X-2023-10-2-30-41
53. Tavan M, Hanachi P, de la Luz Cádiz-Gurrea M, Carretero AS, Mirjalili MH. Natural phenolic compounds with neuroprotective effects. Neurochemical Research. 2024;49(2):306–326. https://doi.org/10.1007/s11064-023-04046-z
54. Quesada-Vázquez S, Eseberri I, Les F, Pérez-Matute P, Herranz-López M, *et al*. Polyphenols and metabolism: From present knowledge to future challenges. Journal of Physiology and Biochemistry. 2024;80(3):603–625. https://doi.org/10.1007/s13105-024-01046-7
55. Chernukha IM, Polishchuk EK, Trubina MV, Nesterova MD, Kupaeva NV. Animal and plant by-products as a source of valuable biologically active substances: A review. Vse o myase. 2025;(1):48–57. (In Russ.) https://doi.org/10.21323/2071-2499-2025-1-48-57

56. Mihaylova D, Dimitrova-Dimova M, Popova A. Dietary phenolic compounds–Wellbeing and perspective applications. International Journal of Molecular Sciences. 2024;25(9):4769. https://doi.org/10.3390/ijms25094769

57. Burakova LN, Shkolnikova MN, Plotnikov DA. Composition and technology development of a complex adaptogenic food additive for the arctic zone population of the Russian Federation. Food Industry. 2023;8(1):32–42. https://doi.org/10.29141/2500-1922-2023-8-1-4

58. Agarkova EYu, Belyakova ZYu, Kondratenko VV. Principles of formation of modular technologies of enteral nutrition products. Food Metaengineering. 2023;1(3):33–46. (In Russ.) https://doi.org/10.37442/fme.2023.3.26

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