

# MEDICINAL PLANTS

## CHEMICAL AND MEDICOBIOLOGICAL PROPERTIES OF CHAGA (REVIEW)

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Data on the chemical composition and pharmacological activity of chaga (*Inonotus obliquus*) are reviewed. The possible mechanisms of action and factors responsible for the discrepancy of data available in the literature are discussed. The physical and chemical characteristics of melanin (polyphenolic chromogenic humin-like complex present in chaga) and its role in the regulation of physiological processes are considered.

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The curative properties of chaga have been known from ancient times. Despite many ages of use in folk medicine and a broad spectrum of biological action, chaga has found only restricted use in the officinal medicine. Numerous attempts to elucidate the therapeutic potential of chaga preparations for the prophylaxis and treatment of cancer and other disorders gave rather ambiguous results. This review presents an attempt at systematizing available published data on the chemical nature of components isolated from chaga, their pharmacological activity, and the mechanism of biological action.

According to the botanic classification, Chaga – also known as birch fungus (*Fungus betulinus*) – represent terrestrial polypore fungi *Inonotus obliquus* (Fr.) Pil of *Polyporaceae* (or *Gymenochaetaceae*) family belonging to *Basidiomycota* phylum (basidiomycetes) [1, 2]. About 25000 species of basidiomycetes have been described, including about 500 polypore fungi that are widely occurring in Europe, Asia, North America, and Africa. However, only a small fraction of these species have been evaluated with respect to biological properties, of which 75% show strong antimicrobial activity, and these may constitute a good source for developing new antibiotics. These fungi contain numerous components exhibiting antiviral, cytotoxic, immunomodulatory, antidiabetic, and antioxidant properties. In Japan, some of the protein-bound polysaccharides from polypores and other basidiomycetes have found their way into the market as anticancer drugs [3].

In Russia, Poland, and Belarus, medicinal preparations are more frequently made of chaga (birch fungus) [1, 2, 4], which represents a product of the fruitless (sterile) stage of development of a wood-destroying fungus parasitizing on trunks, mostly of birch and less frequently of alder, witchen, and bird cherry. Chaga is a shapeless formation [2, 4], differing from the fruiting body (basidiocarp) of a polypore that typically has a hoof shape. The cycle of birch fungus development and the formation of chaga have unique features. Basidiospores of *Inonotus obliquus* spread in air to fall onto damaged sites of the crust of living birch trees (frost-induced damage, broken branches, etc.), grow into the wood, and form a mycelium that consumes the plant juice. The threads (hyphae) of mycelium penetrate into the wood, destroy it, and lead to the formation of a structureless white touchwood. Simultaneously, a fruiting body (basidiocarp) producing spores is formed under the crust (at the sites of initial penetration). On the fourth year, this mycelium emerges at the crust surface, where the fruitless mycelium develops that forms slowly growing, black shapeless overgrowths. After 10 – 15 years of parasitism, these formations become 0.5 – 1.5 m long and 10 – 15 cm thick, weighing up to 5 kg and above. The shape can be either rounded or extended along the trunk, depending on the initial crust damage. These very overgrowths are called chaga. The fungus gradually destroys the trunk, which leads to the breakage and loss of this birch tree. Then, the fungus develops the fruiting body comprising a flat structure of hyphae under the crust. When the crust strips away, the spores fall off in air and are spread with the wind to repeat the cycle [1, 2, 4].

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Chaga has to be collected only from living or freshly cut old birch trees. On dry standing and fallen trees, chaga is destroyed and the content of useful substances drops sharply. The overgrowth is cut off the trunk and the inner loose part is removed. The quality of the raw material, its curative efficacy, and technological properties depend on correct collection and storage. The ambiguity and discrepancy of data about the pharmacological properties of chaga, which have been published by various authors, are explained to a considerable extent by the use of (i) fruiting bodies instead of true (sterile) chaga and (ii) low-quality material taken from the bottom part of old standing trees or from fallen trees. It should be also noted that chaga collected in ecologically unsafe regions (near big industrial centers, highways, etc.) can be dangerous [2, 4, 5]. For example, the samples of chaga collected in the Kemerovo region contained a considerable proportion of heavy metals (lead, arsenic, strontium) [5].

#### USE OF CHAGA IN FOLK HERBAL MEDICINE AND IN HOME

In the folk medicine, chaga is also called “black birch fungus” or “birch lip” (in old Slavonic, “gaga,” which probably explains the origin of word “chaga”). Chaga tinctures were among the most popular preparations in the North and Middle Russia, which were used as remedies for the prophylaxis and treatment of gastric disorders and even cancer. According to legends, the Russian duke Vladimir Monomach was cured from lip cancer by birch fungus [6, 7]. Chaga (in combinations with other herbs) was used for the treatment of gastric and duodenal ulcers and various gastritis. Under forest conditions, “chaga tea” can be used for the treatment of stomach upsets and intestinal pains. Such a decoction is especially popular among hunters and foresters, since this drink assuages hunger, removes tiredness, refreshes, improves general tone, and increases work capacity. In some regions where the population widely consumes such infusions instead of usual teas, the available statistics shows a lower incidence of cancer as compared to the adjacent regions.

Chaga tea is used as a means of increasing the general tone. Patients are frequently recommended to use chaga extracts when it is necessary to reduce the arterial or venous blood pressure. Chaga infusions are also used for the treatment of periodontitis, eczema, dermatitis, and psoriasis. Inhalations of chaga with other herbs reduce inflammations in the nasopharynx and facilitate breathing. Abrasions, scratches, and cuts can be treated with powdered chaga to arrest purulent wound development [6 – 8]. Chaga is nontoxic, well tolerated, and has virtually no counterindications for medicinal usage.

Chaga is also used in agriculture, in particular, in animal breeding: adding chaga to the ration of pigs stimulates the growth of piglets and accelerates the weight gain of fatteners. Chaga has also been used as a fertilizer protecting cultured plants from phytophthorosis and as a plant growth stimulator.

It was recommended to add chaga tinctures in canned preserved vegetable and fruit juices [9].

There is constant interest in chaga preparations in both folk and officinal modern medicine, where chaga is both used alone and enters into numerous multicomponent preparations for internal (decoctions, infusions) and external (ointments) usage. There are patented compositions containing chaga, which are claimed to produce general immunopotentiating and strengthening, antiinflammatory, and antitumor properties and are intended for the prophylaxis and treatment of gastrointestinal diseases, the postoperation treatment of malignant neoplasms, and the therapy of chronic fatigue syndrome [10 – 16]. Obviously, this broad spectrum of pharmacological activity implies the presence of a rich complex of biologically active substances in chaga preparations.

#### CHEMICAL COMPOSITION OF CHAGA

For the first time, the chemical composition of chaga was studied by Dragendorff as long ago as in 1864. However, since he did not find the anticipated active substances (glycosides and alkaloids), the interest in this natural material was lost. Only about one hundred years ago some Russian researchers, including P. A. Yakimov, A. A. Shivrina, E. V. Lovyagina, E. G. Platonov and others [17 – 28] carried out a sufficiently thorough analysis of chaga and the related concentrated extract in comparison with other polypore fungi. It was established that the chemical composition of chaga significantly differs from that of other polypores. In particular, chaga is characterized by a 2 – 3 times greater content of ash elements and a 4 – 12 times smaller content of nitrogen and cellulose than the fruiting body of same polypores [17 – 19]. The ash content in chaga samples varied from 12 to 15%, depending on the site of occurrence. The predominating ash components were potassium oxide (50%), sodium oxide (9 – 13%), and manganese oxide (1.2%). The sol composition of the concentrated extract mostly corresponded to that of the initial material, but the relative content of ash components increased to 23 – 30 of the dry weight. The elemental compositions of chaga and concentrated extract included calcium, silicon, iron, magnesium, zinc, copper, aluminum, phosphorus, and sulfur.

Later, the data of x-ray fluorescence in combination with gravimetric analysis and atomic-absorption spectroscopy showed the following typical composition of chaga: carbon (39%), potassium (9 – 10%), hydrogen (3.6%), nitrogen (0.4%), magnesium (0.64%), calcium (0.37%), chlorine (0.33%), phosphorus (0.23%), sodium (0.05%), rubidium (0.04%), sulfur (0.02%), manganese (0.02%), some minor elements (iron, copper, zinc, vanadium, chromium, and traces of nickel, selenium, iodine, barium, bromine, and strontium, but absence of cobalt, lead, cadmium, mercury, and arsenic). The elemental composition of a dry extract corresponded to that in the initial raw material, except for (i) a threefold increase in the content of magnesium and (ii) the appearance of

cobalt and lead traces (our unpublished data). Differential calculations indicate that the content of oxygen in chaga is about 40–45%, which indicates that oxygen-containing compounds predominate in this complex. The measurements of the optical emission spectra of the samples of chaga collected in the Kemerovo region and Tuva Republic showed the presence of boron, aluminum, silicon, titanium, zirconium, molybdenum, and silver (traces) [5].

Nitrogen in chaga has mostly a protein nature. The products of hydrolysis reveal 15 amino acids, predominantly glycine, aspartic, and glutamic acids (about 40% of total amino acids), tyrosine, serine, threonine, leucine, methionine, lysine, and histidine [20]. Chaga contains flavonoids (flavones, flavonols, anthocyanins, and catechols, including apigenin, naringenin, coryne, and quercetin), triterpene and sterols (6–8%), acid-resistant lignin (25–30%), cellulose (~2%), hemicellulose (~12.5%), pteroylglutamic acid, and some organic acids (acetic, butyric, and oxalic) [18, 21–25].

Another important feature of chaga is the large content (up to 40% per dry weight) of water-extractable substances, of which more than half (50–60% of dry extract residue or 25% of the initial dry material weight) enter into the so-called pigment or chromogenic complex (ChC). This complex is isolated from aqueous extracts in the form of a flocculent precipitate upon acidity change by hydrochloric acid additions from pH 5.5 to 2.5 [18]. It was suggested that the remaining solution contained some mineral salts, hemicellulose, and water-soluble nonreduced polysaccharides (~5%) possessing weak gel-forming properties, which yield reduced sugars upon the subsequent hydrolysis. According to our data, the solution remaining upon ChC precipitation also contains polyphenolic substances such as flavonoids and tannins (up to 15%). It should be emphasized that the high content of ChC is an important distinctive feature of chaga as compared to the other polypore fungi (where the ChC content typically varies within 0.2–2% [18]).

The ChC composition is still insufficiently clear despite the effort of many researchers. It was established that ChC contains trace amounts of ash (0.5–0.2%) and nitrogen-containing compounds (0.5–0.6%), virtually all nitrogen entering into the r-extractable fraction. It was found that ChC forms a colloidal system of intense dark brown color in aqueous solutions at pH 5.5 and can be deposited under the action of mineral acids, electrolytes, and neutral salts, precipitated with lead acetate, and adsorbed on an activated charcoal. Freshly precipitated ChC is soluble only in alkali and sodium carbonate solutions, 50% aqueous ethanol, and 80% aqueous acetone solutions, while being insoluble in all other organic solvents. When dried in air at elevated temperature, ChC almost completely loses solubility, while when dried in vacuum it retains only solubility in alkali and sodium carbonate solutions. Aqueous ChC solutions (especially neutral) are capable of spontaneous oxidation on standing in air, with the formation and gradual precipitation of strongly colored (up to dark-brown) products of the oxidative hydrolysis of ChC

[25]. Chromatographic analysis of the products of acid and oxidative hydrolysis of ChC revealed the presence of aromatic acids with polyphenol structures (syngic, vanillic, pyrocatechuic, pyrogallic, and *para*-benzoic acids) [19, 26]. A specific feature of these acids is the extremely high susceptibility to oxidation and condensation with the formation of dark-brown products, which proceeds with the absorption of 500–600 mg of oxygen per gram of dry substance weight. The oxidation of ChC requires about 350 mg of oxygen per gram of dry substance, which is also indicative of the high reducing ability of this complex [19, 25].

The active components of chaga were found to stimulate the activity of catalase and proteolytic enzymes of the blood. These properties of chaga are close to those of the “respiratory” (in the terminology of V. I. Palladin) plant chromogens, which are capable of restoring the enzymatic activity suppressed by inhibitors [25]. Based on the experimental results it was suggested that ChC is formed from lignin residues and the products of humification of the birch wood. As is known, the wood-destroying fungi (such as *Inonotus obliquus*) cause corrosive decay of wood and they more strongly decompose lignin than cellulose. Lignin molecules decay into simpler compounds via the oxidation of methoxy groups to carboxy, followed by condensation of the oxidation products. According to this hypothesis, ChC is a product of the secondary biosynthesis in the fungus, which yields a highly condensed system of the humic acid (HA) type. However, in contrast to HA, ChC contains almost no nitrogen and is soluble in water. Data available on the HA synthesis are indicative of the participation of microorganisms in the decomposition of plant residues. According to Trusov, this is a multistage process involving the oxidation of aromatic compounds to quinones, their condensation, and the conversion into dark-brown product. These transformations proceed with the participation of the fungal enzyme laccase (*p*-diphenol-oxidoreductase) catalyzing the polymerization process.

The role and physiological significance of ChC for the fungus are still unclear. It was suggested that this complex binds and neutralizes the vast amount of sol elements accumulated by the fungal mycelium during its long-term functioning. In addition, ChC is probably also involved in the metabolism. This readily oxidizable complex can participate in redox processes in the mycelium. Similar to HAs, ChC is a multifunctional macromolecular (polymeric) system appearing in plants or their tissues as a “factor of resistance” under unfavorable conditions accompanying the host–parasite interaction. According to V. P. Filatov, the group of substances activating the suppressed enzymatic activity is called “biogenic stimulants.” These compounds favor the restoration of pathologically violated exchange processes in the organism. Features in common between Has and ChC are the rich micro- and macroelement composition (K, Mg, Ca, Fe, Si, Al) and the broad spectrum of biological and physiological action, including a high antitoxic potential and control over the enzymatic processes. These properties make possible the use of Has and ChC as safe, nonspecific protective

agents increasing the resistance of the organism to the action of unfavorable factors. ChC contains up to 5% methoxy groups, which is intermediate between peat HAs (1.2%) and birch-tree lignins (15.7%), thus confirming the hypothesis concerning the lignin nature of ChC [27–30]. The polyfunctional character of HAs and ChC of chaga is probably related to the polydisperse structure and variable composition of the functional groups and the presence of numerous mineral components [25, 26, 30].

Analysis of the physicochemical properties of macromolecular polyphenolic pigments of chaga allow these substances to be classified as the natural polymer pigments melanins [31, 32]. Melanins constitute the group of high-molecular-weight black and brown pigments formed as a result of the oxidative polymerization of phenols. From the physicochemical standpoint, melanins are heteropolymers – the only known natural polymers with strongly developed polyconjugated bond system [33, 34]. Chemically, melanins are characterized by a specific reactivity that is related to the presence of numerous paramagnetic centers. The paramagnetic centers of melanins are highly stable (in contrast to the labile free radicals of metabolic origin) and are probably involved in the deactivation of labile radicals formed during the exposure to UV or other ionizing radiation. The radioprotector action of melanins has been experimentally confirmed [33] and is now commonly accepted. Melanins are widespread in both the animal and plant worlds. They are found in skin, hair follicles, pigment epithelium of eye retina and iris, brain and spinal marrow, adrenal medulla, and internal ear. The production of melanins drops with increasing age, which gives rise to disorders in some physiological systems of the organism.

In the animal organism, melanins are involved in DNA repair, respiratory chain functioning (as electron acceptors), cell metabolism (as modulators), and photo- and radioprotector systems. The presence of stable free radicals in melanins, as well as the pronounced semiconductor properties and the high susceptibility to enter redox processes, provides protection of the organism under extreme conditions. Indeed, melanins are universal protectors against carcinogenic and mutagenic factors [33–36]. In the hydroquinone form, melanin is a strong chelate-forming agent. Being semiconductors, melanins can catalyze biochemical reactions. The presence of quinone hydroquinone, and carboxy groups in the structure of melanin accounts for its electronic and ion-exchange properties [33, 35, 36]. The melanin character of pigments isolated from chaga was confirmed by the complex analysis of their physicochemical properties. Indeed, these pigments possess a characteristic molecular weight (57 kDa) and the typical optical absorption in the UV-VIS spectral range. The IR spectroscopy data confirmed the presence of the main functional groups, while the EPR data confirmed the presence of stable paramagnetic centers [37]. The pathways of melanin synthesis in *Inonotus obliquus* culture have been studied [31, 38]. It was established that melanogenesis is stimulated by copper ions (0.008%), pyrocatechol (1 mM),

and tyrosine (20 mM). The melanin production is correlated with the synthesis of *o*- and *p*-diphenol oxidases. A comparative investigation of the properties of pigments present in natural chaga and in the material grown in a mycellar culture revealed certain distinctions. The latter pigments contain a greater amount of nitrogen than the former (6.4 against 0.7%), represent the products of tyrosine polymerization, exhibit additional signals in the EPR spectrum, and probably have substantial structural differences (evidenced by thermolysis). For this reason, pigments obtained from the cultured fungus are classified as eumelanins (black pigments of animal origin) and pigments isolated from natural products, as allomelanins (black-brown pigments of higher plants and fungi). Allomelanins belong to polyphenolic condensed biopolymers with quinoid structures of the naphthalene or catechol type [34]. Depending on the conditions, these melanins can occur in the form of phenoxy or semiquinone radicals capable of interacting with the products of xenobiotic oxidation, heavy metals, and other pathological factors.

Unique features of melanin pigments are their stable free radical state and a high content of paramagnetic centers involving carboxy and carbonyl groups. Fungal melanins (in particular, those of chaga) exhibit high antioxidant and gene-protecting properties. Such melanins decreased the genotoxic action of carcinogenic aminobiphenyls (metabolites of benzidine and its derivatives), thus preventing DNA damage and favoring increase in the rate of DNA repair [34, 38]. Both chaga extracts and cultural media exhibit antimutagenic action on uterine cervical carcinoma cells (mostly in the M, G1, and G2 states) and increased catalase activity. It was shown that the antimutagenic activity of mycelial pigments in these cells was not related to the stimulation of catalase activity [39, 40].

The broad spectrum of biological activity of melanins is related to certain features in their chemical structures. The photo- and radioprotector activity is due to the nonradiative absorption of radiation by polyconjugated systems in the entire frequency range. Melanins effectively suppress the peroxidation of polyunsaturated fatty acids and prevent single-strand rupture in DNA, damage of biomembranes, and oxidation of SH groups in proteins and glutathione. As a result, melanins are capable of accelerating the rate of operational wound healing and exhibit immunomodulatory, antiinflammatory, enterosorbent, and antitumor properties in combination with low toxicity [35–41].

The fraction of sterols and triterpenes isolated from chaga contains lanosterol (a tetracyclic triterpene derivative), inotodiol (triterpene alcohol), ergosterol, and some other compounds. These substances are insoluble in water but exhibit partial emulsification and, hence, can be isolated with hot water (up to 80% are retained in the residual material). Inotodiol showed a significant antitumor activity, while lanosterol showed a relatively weak effect [42].

It was reported [43] that lectins were also identified in chaga extracts. Lectins (or agglutinins) belong to the class of

complex glycoproteins, some of which may contain calcium, magnesium, and other ions [44]. These centers can reversibly bind carbohydrates and participate in their transport and deposition (thus producing a hypoglycemic action and reducing sugar in the blood of diabetes patients). It was also found that lectins can stimulate the growth and multiplication of lymphocytes, participate in the regulation of immunochemical reactions, and block the receptors of tumor cells, thus suppressing their migration [43, 44].

Thus, chaga possesses a complex of important biologically active substances, which can participate in the regulation of metabolism and in the correction and prophylaxis of pathologic disorders. The main problem is to retain these substances in the course of material collection and processing, to make high-quality preparations, and to determine the optimum schedule of their administration.

## MEDICOBIOLOGICAL PROPERTIES OF CHAGA

### *Safety of Chaga Preparations*

Lazovskaya [45] tested a chaga extract on experimental animals of four kinds (mice, rabbits, dogs, and cats) and showed that the preparation was well tolerated in large doses: LD<sub>50</sub> for mice was 6.5 g/kg body weight. The extract administered *per os* in doses up to 1.0 g/kg did not cause any change in the behavior of rabbits, dogs, and cats. Greater doses sometimes led to toxic effects, which were manifested by movement disorders and motor paralysis. In order to elucidate the factors responsible for this behavior, a special test was performed on a frog with ligated femoral artery, in which the sciatic nerve was irritated after drug administration. It was established that the paralysis was caused by suppression of the CNS rather than the peripheral nervous system [45]. In a chronic administration test, rats and rabbits received a chaga extract in a daily dose of 1 and 0.3 g/kg body weight over a period of 5–6 months. After this period of time, no significant changes were observed in the state of extracted internal organs. The only deviations from normal development were (i) a somewhat less intense growth of rabbits during the first three weeks of treatment and (ii) a lower level of fat deposition in subcutaneous tissue, omentum, and adipose capsules of test rats as compared to the control animals. Based on these results, it was concluded that chaga is well tolerated in the indicated doses and does not exhibit accumulated toxic effects. The same tests also showed no evidence of pyrogenicity upon peroral administration of chaga in rabbits [45, 46].

Purified chaga preparations exhibited no toxicity upon parenteral introduction in doses corresponding to (0.1–5)-fold daily therapeutic dose for humans [47–50]. No changes in the behavior and weight of test animals (rabbits and white mice) were observed in the tests for chronic toxicity of the precipitated chaga preparation administered via an intragastric tube in a daily dose of 0.2–1 g/kg (rabbits) and 5 g/kg (mice) over a period of 10 days [51]. The

complete safety of chaga preparations administered in therapeutic doses was also confirmed by numerous clinical tests with continuous many-year administration in patients with stage IV cancer, gastrointestinal ulcers, etc. [11, 12, 52–54].

### *Antitoxicant, Radioprotector, and Adaptogen Action of Chaga*

Experiments performed *in vitro* on the fermentation of baker's yeasts showed that chaga preparations decrease the toxic action of sodium fluoride, which is a strong inhibitor of catalase, enolase, and esterase (enzymes catalyzing the fermentation process) [55]. The antitoxic, recovering effect of chaga was also observed in experiments with the germination of wheat seeds pretreated with copper sulfate [55].

The results of tests *in vivo* showed that the administration of chaga with meals in white mice led to a decrease in the level of liver atrophy caused by carbon tetrachloride [56]. However, the growth of connective tissues was still more pronounced than that in the control. The extract of chaga introduced via a gastric tube produced antitoxic action in mice injected with the cytostatic ethymidine in a lethal dose (30 mg/kg, *i.p.*). The survival of test mice was significantly greater (65%) than that in the untreated control group (5%). However, no protective effect of chaga was observed in the group of animals that received a chaga extract with drinking water [57].

The administration of chaga in the form of a freeze-dried cryopowder in Wistar rats 2–3 min after the intravenous injection of a radioactive <sup>90</sup>Sr isotope preparation led to a decrease in the extent of radionuclide deposition in the bone and soft tissues of animals. The amount of radionuclide eliminated with urine also exhibited a reliable (33–35%) increase [58]. The daily administration of this cryopowder over the first 30 days during long-term (up to 60 days) gamma-irradiation of Balb/c mice led to an increase in the average survival life of animals up to 305 days (against 186 days in the untreated control group) and prevented a sharp drop in the leukocyte number, slowed the development of leukopenia, and restricted the rate of lipid peroxidation to a level close to that in the intact control [58]. The rate of recovery processes in hematopoietic tissues (bone marrow cells) was also somewhat higher in the test groups receiving chaga than in the uncured control. The moderate intensity of protein synthesis and body weight gain showed evidence for the adaptogen action of chaga in irradiated rats [59].

Rudakov [60] described the effect of a chaga extract on the development of resistance to unfavorable external factors in white mongrel mice exposed for 13–20 min to a high temperature (70°C). The extract was administered via a gastric tube in the test animals 1 h before the onset of heating. The results were evaluated in terms of percentage survival, which increased from 30% in the untreated control group to 75% (at the end of test) in the group treated with chaga; on a longer time scale (48 h), the survival in these groups increased from 7 to 60%, respectively. A preliminary administration of chaga over 5 days before test also led to a (2–2.5)-fold increase in the resistance of mice to elevated

temperatures (relative to the untreated control), depending on the dose. The test animals pretreated with chaga tolerated better the increased temperature (exhibited higher activity and moved, in contrast to the untreated control). However, the survival of mice in the group treated with chaga at an increased dose (0.5 ml per mouse) was 10% lower than that in the control group. Thus, the biologically active complex of chaga produces a stimulating action on the animal organism, improves its response, increases resistance, and normalizes physiological functions like the other biogenic stimulators [60].

The biostimulating action of chaga is dose-dependent: extracts are most effective in small doses and may even lead to the opposite action in elevated doses. For example, chaga tinctures diluted in the range from 1/2000 to 1/3000 exhibited a stimulating action on the development of infusoria (simplest unicellular species), while leading to their loss at an increased concentration (1/100 to 1/1000 dilution) [61]. Analogous results were obtained for the influence of chaga on the germination of wheat seeds [62], according to which chaga extract solutions stimulated the growth process in the range of concentrations within 0.01 – 0.00001% while inhibiting the growth at concentrations within 0.1 – 0.5%. At the same time, the products of hydrolysis (aromatic acids) of the polyphenolic ChC always acted as inhibitors irrespective of the concentration [62]. This is probably one of the factors explaining the contradictory data reported by various authors on the activity of chaga.

#### *Effect of Chaga on Physiological Functions*

According to the results of ECG and pneumography measurements in the experiments on rabbits, chaga favorably influences cardiac activity by increasing the contractility of the myocardium and by calming the heart rhythm. The administration of chaga improved the state of the vegetative nervous system, in particular, with respect to control over the cardiovascular and respiratory systems [47, 48, 50]. In particular, Berezina et al. [50] established that chaga accelerated restoration of normal nerve function suppressed by calcium chloride [50]. Therefore, purified chaga preparations are potential stimulators capable of restoring the active state of nerve tissues [50]. In experiments performed on an isolated frog heart [63], a small (~0.01%) concentration of chaga produced variations in the cardiac activity: an initial short-term small decrease in the systolic amplitude was followed by its increase (without a change in the heart rhythm). Then, the heart contractions became more powerful and were accompanied by increasing diastole filling. The heart increased in volume, which led to still stronger contractions of the myocardium and to an increase in the systole volume. In this regime, the heart exhibited stable functioning over the entire observation period (3 – 5 h). It was suggested that chaga influenced the nervous apparatus of the heart. The same effects, but in a more pronounced form, were observed for the same chaga preparation in higher concentrations (0.02 and 0.05 %). However, an increase in the chaga concentration above 0.1% led to a rapidly developing decrease in the heart

rhythm (caused by an increase in the diastole and the pause duration) and the appearance of arrhythmia.

In experiments performed *in vivo* [63], small (~0.01%) concentrations of chaga did not influence frog cardiac muscle functioning, while higher concentrations (0.02 and 0.05%) induced variations in the heart operation. The initial short-term decrease in the systole amplitude and in the heart rhythm was followed by a significant growth in the amplitude and by an increase in the heart rhythm, which implied improvement in the tone of sympathetic innervation. Then, the heart rhythm somewhat decreased but the amplitude remained high and the heart contractions became more powerful than before the introduction of chaga. Both the atrium and ventricles exhibited intense contractions and the heart filling was improved. Increased working capacity of the heart was observed for several hours. However, still higher concentrations of chaga led to a decrease in the amplitude and sometimes to the development of arrhythmia. It was concluded [63] that chaga influenced the cardiac activity via the central nervous system, the effect being dependent on the drug dose and the functional state of the cardiac muscle and the central nervous system.

Experiments performed with a deposited pigment complex (instead of the whole chaga extract) showed evidence for a milder action, involving neither suppression of the heart function nor the development of arrhythmia. The optimum concentrations (0.5 – 1.0%) of this complex produced a trophic action on the cardiac muscle (additionally increased the power and amplitude of contractions) and increased the tone of vegetative innervation. This phenomenon was related to the increased lability of the apparatus of heart innervation and the sensitivity of the heart to the nerve pulses transmitted via the extracardiac nerves [64].

Golovko [65] studied the effect of bioglucans isolated from birch tree on the electrical activity of cells from the frog heart venous sinus. It was established that a 0.0001% solution improved the excitability properties of cell membranes, the effect being comparable with that of a hypocalcium solution (except that the positive chronotropic action of bioglucans was 50 – 100 times longer). The encephalograms of the cerebral cortex of rabbits measured before and 20 – 39 min after the intramuscular introduction of a sterile chaga extract [66] clearly revealed an increase in the spontaneous bioelectric potential in the cortex (especially pronounced in the occipital region), which was interpreted as indicative of the effect of chaga on the metabolism in the brain cortex cells.

#### *Antioxidant and Immunomodulatory Properties of Chaga*

The antioxidant properties of chaga have been recently studied using the method of spontaneous and initiated (divalent iron) chemiluminescence [67]. It was established that chaga produced a 1.75-fold decrease in the spontaneous optical emission and a 2.5-fold decrease in the induced emission intensity, which was comparable with the effect of ionol (a reference antioxidant) at a concentration of 0.01 mM [67].

The antioxidant action of chaga was confirmed under clinical conditions by the characteristics of lipid peroxidation in patients suffering from pneumonia [67] and soft arterial hypertension [68]. According to the results of these investigations, the effect of chaga was comparable with that of the well-known antioxidants – vitamins C and E. More recently, it was reported [58] that a freeze-dried chaga crypowder prevented the growth of malonic dialdehyde (MDA) concentration in the blood plasma of rats against the background of a 60-day gamma irradiation and reduced the level of P-proteins in the blood 3.5 times relative to the untreated control [58]. The MDA determination is a highly sensitive test for destructive oxidation processes, destabilization of the immune protection system, and homeostasis violations in the organism.

Gaponenko [69] studied the antioxidant and immunomodulator properties of a chaga-based balm (Berezka) under experimental and clinical conditions. It was established that this preparation normalized the content of MDA and SH groups and the activity of catalase and superoxide dismutase in the blood plasma (violated by carbon tetrachloride injections) and stimulated the immune response to vaccination [69]. The immunomodulator effect of a chaga preparation (BRM-Ch) was also studied on mice gamma-irradiated to a dose of 3.0 Gy [70]. One month after irradiation, the group of animals treated with chaga for seven days immediately after the exposure showed evidence for a correction of the content of immunocompetent cells. This was manifested by an increase in the percentage content of T-helper lymphocytes from  $7.8 \pm 0.9$  to  $16 \pm 2$  and by normalization of the cytotoxic activity of natural killers. In the group of animals treated with chaga for two weeks after irradiation, normalization was observed in the content of both T-helper lymphocytes and lymphocytes containing membrane receptors. The activity of natural killers reached a level characteristic of intact animals [70].

The results of our tests on Balb/c mice also showed the broad spectrum of immunotropic activity of chaga extract. In a dose range within 1 – 100 mg/kg, chaga produced a (1.5 – 3.5)-fold increase in the proliferative activity of splenocytes transformed *in vitro* by a polyclonal Con A mitogen or an alloantigen in a mixed culture of lymphocytes (MCL) from an allogenic mice spleen. Chaga induced the formation of additional cytolytic T-lymphocytes (T-killers) in the MCL. Long-term (up to 8 weeks) peroral administration of chaga stimulated the cytotoxic activity of peritoneal macrophages. It was established that chaga makes possible the correction of secondary immunodeficient states caused by the administration of an antitumor cytostatic aranose in mice.

#### *General Strengthening Action of Chaga*

Chaga, befugin (dense chaga extract with cobalt salt additives), and chaga tinctures were allowed by the Ministry of Public Health of the former USSR as medicinals for the treatment of chronic gastritis, gastrointestinal tract

dyskinesia with atony manifestations, and gastric ulcers and as a symptomatic remedy improving the general state of oncological patients. Chaga produces a general tone-increasing and pain-relieving action [71]. Chaga preparations are especially effective in combination with the traditional methods. The inclusion of chaga into a scheme of therapy for patients with gastric ulcers decreased their stay in clinics from 42 to 30 – 35 days, while the duration of remission in patients treated with chaga was 1.5 – 2 times longer than in the analogous cases treated using other methods without chaga administration [72]. It was reported that chaga made possible the successful treatment of neglected psoriasis in a group of patients simultaneously suffering from gastrointestinal disorders. After continuous administration of chaga for two to three months, complete remission was reached in 38 patients and an improved state was observed in 8 patients [73].

It was reported [54, 74, 75] that long-term administration of chaga significantly improved the general condition and objective state of patients with incurable stage III – IV cancer, irrespective of the tumor location. In most of these patients without pronounced cachexia, a three- to four-week administration of chaga led to a decrease and termination of the pain syndrome, which allowed the administration of narcotic drugs to be stopped.

At present, the list of registered biologically active additives containing chaga includes Chagovit (capsules and elixir), Extrabesungin (drajee), Berezka (balm), Litovit Ch (tablets), dry chaga extract, freeze-dried chaga crypowder, and some others. These preparations by no means offer an alternative to traditional drugs, but have to be considered as important components for various combined therapy schemes ensuring accelerated restoration of protective forces and improvement of the general condition of patients prior to, during, and after the treatment of serious diseases. Chaga preparations alone or in combinations also offer effective means of prophylaxis.

This brief analysis of published data shows that chaga is among the most interesting domestic sources of biologically active substances. Unfortunately, this material is still insufficiently studied and the available data are rather contradictory. Although chaga does not possess specific antitumor and cytotoxic activity, long-term administration of extracts improves the general state even of incurable stage III – IV cancer patients (except for cases of extremely pronounced cachexia) irrespective of the tumor location and favor normalization of the physiological functions of the organism violated by the pathological process. The pharmacological action of chaga is apparently mediated by the central and peripheral nervous system and is probably related to the total complex of biologically active substances, primarily to a polyphenolic chromogenic humin-like melanin complex. Evidently, this complex is capable of modulating cell metabolism at the level of the central and peripheral nervous system so as to induce correction of the bioregulation of intrinsic protective systems and resources of the organism. The contradictory character of published data is probably related to

various factors – both technological (different quality of the initial raw materials and final preparations) and clinical (choice of doses, schemes, and regimes of treatment, initial state of patients, etc.).

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