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Computer-aided Evaluation of Polyvalent Medications' Pharmacological Potential. Multiphytoadaptogen as a Case Study

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Abstract: Many human diseases including cancer, degenerative and autoimmune disorders, diabetes and others are multifactorial. Pharmaceutical agents acting on a single target do not provide their efficient curation. Multitargeted drugs exhibiting pleiotropic pharmacological effects have certain advantages due to the normalization of the complex pathological processes of different etiology. Extracts of medicinal plants (EMP) containing multiple phytocomponents are widely used in traditional medicines for multifactorial disorders' treatment. Experimental studies of pharmacological potential for multicomponent compositions are quite expensive and time-consuming. In silico evaluation of EMP the pharmacological potential may provide the basis for selecting the most promising directions of testing and for identifying potential additive/synergistic effects.

Multiphytoadaptogen (MPhA) containing 70 major phytocomponents of different chemical classes from 40 medicinal plant extracts has been studied in vitro, in vivo and in clinical researches. Antiproliferative and anti-tumor activities have been shown against some tumors as well as evidence-based therapeutic effects against age-related pathologies. In addition, the neuroprotective, antioxidant, antimutagenic, radioprotective, and immunomodulatory effects of MPhA were confirmed.

Analysis of the PASS profiles of the biological activity of MPhA phytocomponents showed that most of the predicted anti-tumor and anti-metastatic effects were consistent with the results of laboratory and clinical studies. Antimutagenic, immunomodulatory, radioprotective, neuroprotective and anti-Parkinsonian effects were also predicted for most of the phytocomponents. Effects associated with positive effects on the male and female reproductive systems have been identified too.

Thus, PASS and PharmaExpert can be used to evaluate the pharmacological potential of complex pharmaceutical compositions containing natural products

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1 Introduction

Many common diseases including cancer, degenerative and autoimmune disorders, diabetes and others are multifactorial pathologies.[1,2] Effective treatment of complex diseases requires the engagement of multi-targeted pharmaceutical agents.[3–5] Polypharmacology is often considered in association with undesirable off-target side effects which lead to the failures of drug discovery projects.[6] However, being particularly designed, polyvalent pharmaceuticals hitting several targets involved in pathological process may provide certain benefits in the curation of multifactorial disorders due to the additive or synergistic action of separate components.[3,5,7]

Secondary metabolites (phytocomponents) of medicinal plants are the promising source of novel pharmaceutical substances because they are adopted by Mother Nature for binding with proteins and other biological targets.[8] Recent cheminformatics analyses have demonstrated that natural products cover broader chemical space compared to the drugs and chemical probes of synthetic origin.[9–11] Investigation of separate phytocomponents' pharmacological profiles requires their extraction and purification. In some cases, the extracted individual substances lost the pharmacological action suggested by the folk medicines or shown in biological assays for the whole extract. Thus, despite the numerous issues that may occur with experimental evaluation of medicinal plants' biological activity[12] and concerns regarding their introduction to medical practice[13] herbal phytocompositions are considered as promising remedies for complex disorders' treatment of.[14,15]

Multiphytoadaptogen (MPhA) was developed by the Blokhin National Medical Research Center of Oncology. It is approved in Russia as a parapharmaceutical agent consisting of components from 40 medicinal plants including *Panax ginseng*, *Rhodiola rosea, Eleutherococcus senticosus, Eucalyptus globules, Juniperus communis, Valeriana officinalis, Polygonum aviculare, Leonurus cardiaca*, etc..[16–19] MPhA has been standardized by both analytical and biological methods. With the help of NMR (nuclear magnetic resonance) and UV (ultra-violet) spectrometry the possibilities of controlling the medication by the extracts' composition were determined. With the use of HPLC (high-efficiency liquid chromatography), CMS (gas-liquid chromatography with mass detector), UV-HPLC and HPLC-MS/MS (tandem mass spectrometry) analyses of the MPhA composition many compounds were quantitatively identified – triterpene glycosides, phenylpropanoids, lignans, flavonoids, essential components, vitamins, etc..[20–29] As a result, 70 major phytocomponents have been selected in the MPhA composition. Pharmacological and toxicological characteristics of MPhA have been investigated *in vitro, in vivo* and in clinical studies.[30–32] A wide range of pharmacological effects (adaptogenic, antitumor, immunomodulatory, antimutagenic, etc.) exhibited by MPhA has been demonstrated. It was shown that MPhA corresponds to the class IV safety level according to the OECD classification (practically non-toxic).

No one pharmaceutical agent could be tested in all possible bioassays and against all known targets. Moreover, experimental studies of pharmacological potential of herbal extracts are rather expensive and time-consuming.[33] Thus, the additional information may be obtained using *in silico* approaches which are widely used for the analysis of natural products' properties.[23–37] Computational methods allow identifying the most probable biological activities of the separate phytocomponents, elucidating the putative mechanisms of actions that caused the observed pharmacotherapeutic effects and estimating the potential additive/synergistic effects of the whole phytocompositions.

The computer program PASS (Prediction of Activity Spectra for Substances) is used for biological activity prediction of drug-like compounds for over thirty years.[38–41] Biological activity spectra estimated by PASS may be analyzed by PharmaExpert software[35] which identifies the activity-activity relationships shedding light on probable additive or synergistic pharmacotherapeutic effects. Earlier, PASS and PharmaExpert were successfully applied to illuminate the hidden pharmacological potential of natural products and determine the most promising directions of the further experimental testing.[42–49] However, in those investigations a few phytocomponents of a particular medicinal plant have been analyzed while MPhA represents much more complex mixture of 70 major secondary metabolites from 40 medicinal plants.

In this study we carried out PASS predictions of biological activity spectra for each of 70 phytocomponents, integrated the obtained information using PharmaExpert to estimate the probable additive/synergistic effects, compared the predicted biological activities with known ones and determined the putative mechanisms of action caused by the observed pharmacotherapeutic effects.

2 Materials and Methods

2.1 Chemical Composition of MPhA

70 major chemical compounds included in the pharmaceutical composition of MPhA were used for computer-aided prediction in the present study.

Structural formulae of chemical compounds presented in SDF format were extracted from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Chemical compounds' information including the source plant is provided in Table S1 of Supporting information.

2.2 PASS Software

PASS is "one of the earliest and most widely used examples of data-mining target elucidation" software.[50] Based on the

analysis of structure-activity relationships for the training set included more than 1.5 million of known biologically active compounds, PASS 2020 predicts over 8,000 biological activities. The average of the values of the Invariant Accuracy of Prediction (IAP) calculated by leave-one-out cross-validation procedure is about 0.9301; while in 20-fold cross-validation it was 0.9287. In PASS algorithm naïve Bayes approach is used; structural formulae are represented by MNA (Multilevel Neighborhood of Atoms) descriptors; biological activities are described in qualitative mode (active/inactive). A more detailed description of PASS algorithm is available in publications.[39–41] In this study we have limited the set of predictable activities by 1,945 pharmacotherapeutic effects and mechanisms of action, which are predicted with average accuracy 0.9710 (leave-one-out cross-validation). Very close results (average accuracy 0.9705) were obtained in 20-fold cross-validation (Table S2 of the Supporting information), which demonstrates the good predictivity of the model. Prediction results for each MPhA phytocomponent were obtained as the list of probable activities with two estimates: Pa - probability to be active, and Pi - probability to be inactive. By default, all activities estimated with $Pa > Pi$ are considered to be probable; however, depending on the particular purpose, this threshold may be varied.

2.3 PharmaExpert Software

PharmaExpert[35] provides the set of options for visualization and analysis of PASS predictions. In this study we have analyzed the additive/synergistic effects by a combination of biological activity spectra predicted for 70 MPhA phytocomponents. Using the knowledgebase of relationships between the mechanisms of action and pharmacotherapeutic effects we identified the probable drug-drug interactions leading to the integrative action of the whole MPhA that may increase the activity of separate phytocomponents.

3 Results and Discussion

3.1 Analysis of the Structural Diversity

The chemical diversity of the set of compounds was analyzed using MNA descriptors and Tanimoto similarity estimates. The average value of similarity estimates is only 0.09. However, there are some groups of structures characterized by high similarity. These groups include ginsenosides (Rb1, Rb2, Re, Rc, Rd, Rf, Rg1, Rg2, Ro) with a mean similarity of 0.74, aralosides (A, B, C) with a mean similarity of 0.93 and amino acids (Alanine, Aspartic acid, Arginine, Glutamic acid) with a mean similarity of 0.59. Such composition provides the needed pharmacological effects of MPhA despite the possible variations in quantitative contents of separate phytocomponents. A heat map of the structures similarity is shown in Figure S1 of the Supporting Information.

Such composition provides the needed pharmacological effects of MPhA despite the possible variations in quantitative contents of some phytocomponents. Taking into account the difficulties of determination for individual phytocomponents' concentration in the complex of extracts from 40 medicinal plants, MPhA standardization is provided by biological methods.[20]

3.2 *In silico* Analysis of MPhA Antitumor Effects

Taking into account the known data regarding the MPhA properties, the main categories of probable pharmacological effects were considered: antitumor actions as well as effects associated with improving the duration and quality of life.

It was found that 51 of 56 antitumor effects are predicted for MPhA. The amounts of compounds with additive/synergistic effects in relation to these effects are shown in Figure 1.

As can be seen from the data shown in Figure 1, at the $Pa > Pi$ threshold an antineoplastic effect is predicted for each of 70 compounds, the average Pa–Pi value is 0.56. An antimetastatic effect is predicted for 69 compounds with a mean Pa–Pi of 0.62. For 13 tumors the number of active compounds exceeds 50. The corresponding Pa–Pi values for individual compounds are presented in Table S3 of the Supporting information.

We also performed a literature search for information on laboratory testing for the antimetastatic activity of the compounds discussed in this paper. The results are summarized in Table S8 of the Supporting information. Experimental confirmation of antimetastatic action was found in 26 cases out of 69. Twenty-five of these confirmations were found for compounds with a $Pa–Pi > 0.5$ threshold, representing 41% of all compounds with predicted antimetastatic effect at this threshold. Unfortunately, for the other 43 compounds with the predicted antimetastatic effect we did not find any information regarding their experimental testing on this activity. Thus, at the moment it is impossible to conclude either they are active or inactive. However, the antimetastatic effect of 26 phytocomponents (predicted and confirmed experimentally) is the reasonable justification for the antimetastatic action of MPhA.

PharmaExpert knowledgebase allows revealing the most probable mechanisms of antitumor action. These include antioxidant, apoptosis agonist, caspase 3 stimulant and transcription factor NF kappa B inhibitor. Pa–Pi values calculated for these mechanisms are presented in Figure 2.

According to the PharmaExpert assessments, these mechanisms of action contribute to the development of therapeutic effect against cancer of the stomach, lung,

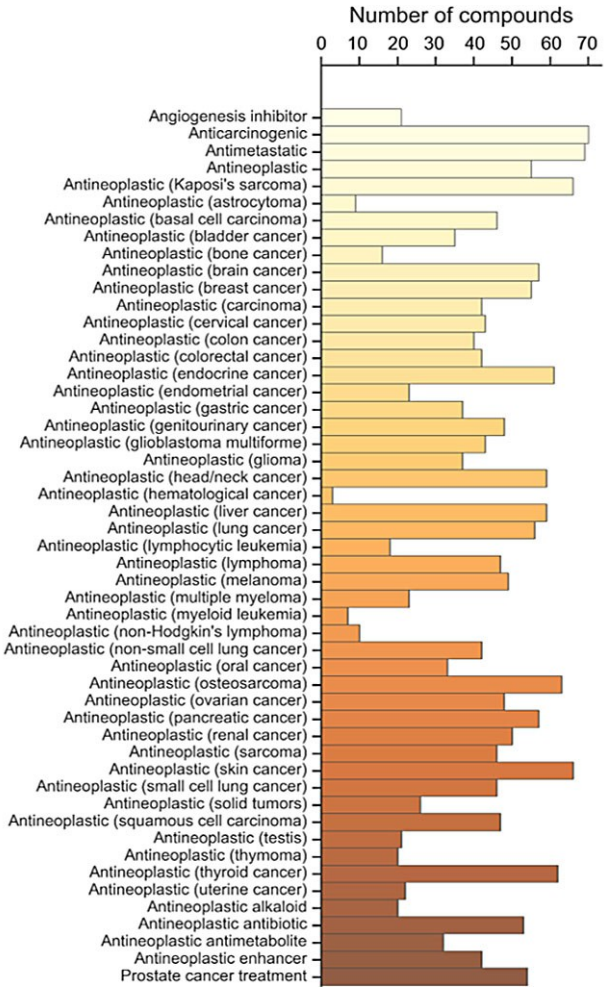

Figure 1. The most pronounced synergistic effects of antitumor activity at the Pa > Pi threshold for the compounds analyzed.

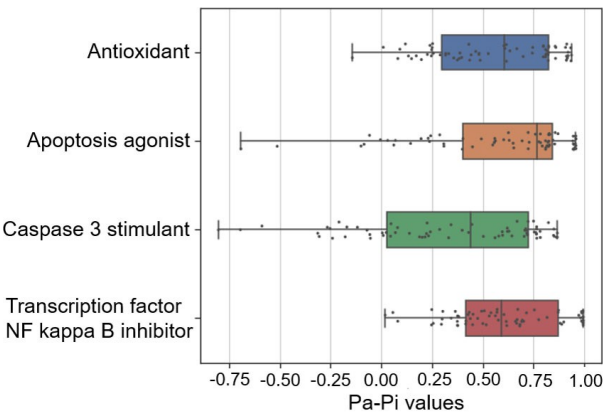

Figure 2. The most pronounced synergistic mechanisms of antitumor activity at the Pa > Pi threshold.

kidney, prostate, cervix, liver and melanoma. Data on the therapeutic effects of MPhA have been confirmed at various stages of investigation. Calculated average Pa–Pi values for the mechanisms of action, associated antitumor effects, and relevant studies are shown in Table 1. Pa–Pi values for individual compounds and antineoplastic effects are presented in Table S3 of the Supporting Information. The Pa–Pi values for the individual compounds and mechanisms of action associated with the antineoplastic effect are presented in Table S4 of the Supporting Information.

Taking into account the key role of the adhesion interactions' problem including immune reactions during the malignant growth,[51,52,53] we predicted the effects of MPhA phytocomponents on associated adhesion mechanisms. The results obtained are shown in Figure 3.

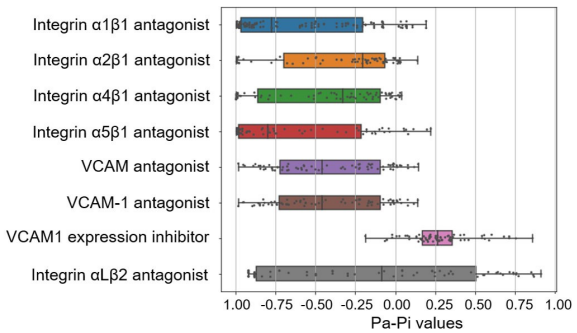

Figure 3. The most pronounced synergistic mechanisms associated with the adhesive effects for MPhA substances at the Pa > Pi threshold.

Figure 3 shows that for antagonism against beta 1 integrins (providing heterotypic adhesion of tumor cells to other tissues promoting metastasis) the positive Pa–Pi values obtained do not exceed the threshold equal to 0.25. Compounds with Pa > Pi scores are predominantly amino acids (proline, tyrosine, arginine, alanine, glutamic acid, and aspartic acid). The highest Pa–Pi values for the agonistic effect on beta 2 integrins expression (providing heterotypic adhesion of tumor cells and cytotoxic lymphocytes - immune effectors) were obtained for the ethyl linolenate (Pa–Pi = 0.85), ethyl isovalerate (Pa–Pi = 0.86), ethyl linoleate (Pa–Pi = 0.86) and ethyl palmitate (Pa–Pi = 0.91).

In the set of compounds studied, there are fatty acids (ethyl linolenate, ethyl isovalerate, ethyl linoleate, ethyl palmitate). According to the interpretation of the results of the prediction, Pa–Pi values calculated for fatty acids and selected adhesion mechanism "Integrin alphaLbeta2 antagonist" exceed the threshold of Pa–Pi > 0.8. The biological activity obtained at this threshold can be characterized as a biological activity with a high chance of laboratory confirmation. The effect of these substances on tumor cell adhesion has been confirmed in the literature.[54] For the remaining compounds and mechanisms of adhesion (except VCAM1 expression inhibitor) values do not exceed the threshold value of Pa–Pi < 0.25, and associations between

Table 1. List of antitumor effects and action mechanisms associated.

Pharmacological effect	Research stage	Mechanism	Mean Pa–Pi value
Antineoplastic (gastric cancer)	*In clinic*	Apoptosis agonist	0.68
Antineoplastic (lung cancer)	*In vivo*	Apoptosis agonist	0.68
Antineoplastic (liver cancer)	*In vivo*	Apoptosis agonist	0.68
Antineoplastic (renal cancer)	*In vitro*	Apoptosis agonist	0.68
Antineoplastic (breast cancer)	Out of trials	Apoptosis agonist	0.68
Prostate cancer treatment	Out of trials	Apoptosis agonist	0.68
Antineoplastic (cervical cancer)	Out of trials	Apoptosis agonist	0.68
Antineoplastic (melanoma)	Out of trials	Apoptosis agonist	0.68
Antineoplastic (gastric cancer)	*In clinic*	Transcription factor NF kappa B inhibitor	0.62
Antineoplastic (lung cancer)	*In vivo*	Transcription factor NF kappa B inhibitor	0.62
Antineoplastic (renal cancer)	*In vitro*	Transcription factor NF kappa B inhibitor	0.62
Antineoplastic (breast cancer)	Out of trials	Transcription factor NF kappa B inhibitor	0.62
Prostate cancer treatment	Out of trials	Transcription factor NF kappa B inhibitor	0.62
Antineoplastic (lung cancer)	*In vivo*	Antioxidant	0.56
Antineoplastic (renal cancer)	*In vitro*	Antioxidant	0.56
Prostate cancer treatment	Out of trials	Antioxidant	0.56
Antineoplastic (melanoma)	Out of trials	Antioxidant	0.56
Antineoplastic (lung cancer)	*In vivo*	Caspase 3 stimulant	0.52
Antineoplastic (renal cancer)	*In vitro*	Caspase 3 stimulant	0.52
Antineoplastic (breast cancer)	Out of trials	Caspase 3 stimulant	0.52
Prostate cancer treatment	Out of trials	Caspase 3 stimulant	0.52

the studied compounds and these mechanisms of action were not found in literature.

The detailed results of the action associated with the adhesive effects mechanisms prediction are presented in Table S5 of the Supporting information.

3.3 Effects Related to the Improvement of Life Quality and Duration

Probable biological activities of MPhA activities reveal the potential effects on the duration and the quality of a patient's life. A total of 15 effects have been observed including chemopreventive, immunomodulatory and anti-inflammatory, radioprotective, as well as anti-neurotic and neuroprotective associated with therapeutic action against Parkinson's disease and its symptoms.

MPhA as an adaptogen has been shown to be effective in preventive oncology. As it is known, preventive oncology includes primary (prophylaxis of the occurrence, or chemoprophylaxis), secondary (prophylaxis of relapses and metastases) and tertiary (prophylaxis of chemo-radiation therapy side effects) prophylaxis of cancer diseases. No doubt, antitumor and protective effects are the main properties that medications for preventive oncology should exhibit. Experimental and clinical studies of MPhA have revealed antimutagenic (which is important for primary cancer prevention, or chemoprophylaxis), antitumor (essential for secondary prevention), radioprotective, hormone-modulating, antioxidant, neuroprotective, immunomodulatory, including adhesive and interferon-inducing effects (which are important for tertiary cancer prevention). All of these activities promote survival and the quality of a patient's life.[32] By the way, adaptogens which are known to promote longevity affect the neuro-endocrine-immune system including antimutagenic, immune- and hormone-modulating, neuroprotective and antiparkinsonian properties.[15]

That is why 15 observed activities relate to the selection of the potential effects on the duration and the quality of a patient's life including chemopreventive, immunomodulatory and anti-inflammatory, radioprotective, as well as anti-neurotic and neuroprotective associated with therapeutic action against Parkinson's disease and its symptoms.

It can be concluded that the model covers antitumor and protective effects, which provide an increase in the duration and quality of human life under conditions of multifactorial environmental influence.

The number of compounds characterized by the most pronounced additive/synergistic effects in relation to the above effects is shown in Figure 4.

As can be seen from Figure 4, for each of 70 compounds at a given threshold a chemopreventive effect (oncoprophylaxis) is predicted. For 17 of them - at a threshold of Pa–Pi $>$ 0.9. For 68 compounds, a radioprotective effect is predicted. The total immunomodulatory effect is predicted for 47 compounds with an average Pa–Pi value 0.4. Interferon-inducing effect is predicted for 34 compounds (the average Pa–Pi value 0.41). NK cell stimulation is predicted for 66 compounds (the average Pa–Pi value 0.42). An anti-inflammatory effect is also predicted for 63 compounds with an average Pa–Pi value 0.41.

Antiallergic effects are predicted for 48, 36, and 15 compounds: Allergic conjunctivitis treatment, Antiallergic

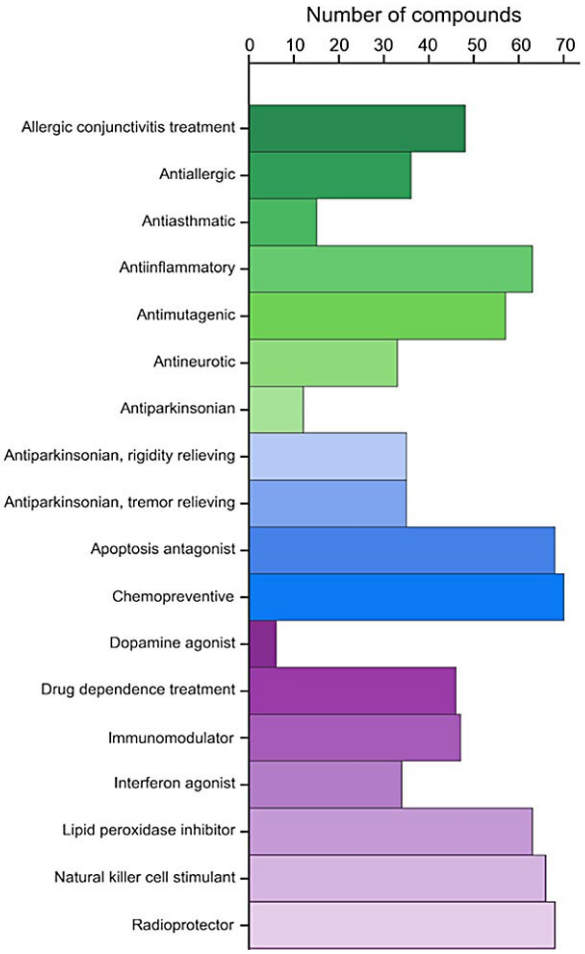

Figure 4. The most pronounced additive/synergistic effects associated with improving the duration and quality of patient's life at the threshold Pa > Pi.

and Antiasthmatic, respectively. For 24, 9 and 2 compounds these effects are predicted at a threshold of Pa–Pi > 0.5.

More information about the obtained Pa–Pi values for the above effects and mechanisms of MPhA action is given in Table S6 of the Supporting information.

3.4 Retrospective Validation of Prediction Results

3.4.1 In vitro *Studies (On Cells)*

It was demonstrated that the adaptogenic activity of MPhA turned out to be higher in comparison with those of the individual phytoadaptogens. The adaptogenic activity was assessed by the accelerated growth characteristics of baker's yeast cells culture *Saccharomyces cerevisiae* using an energetically poor nutrient medium in the presence of the MPhA.[55,56]

The antiproliferative effect of MPhA has been shown *in vitro* on cell cultures of ovarian and cervical human adenocarcinomas as well as on human hypernephroma. However, MPhA did not affect the proliferation of normal kidney embryonic cells of the pig. This characterizes MPhA as a real adaptogen that provides an effect on pathology but not on the normal state. In other words, one may conclude that MPhA has a selective antitumor activity.[57]

The antimutagenic effect of MPhA *in vitro* was manifested in a decrease in the level of spontaneous and induced mutations in yeast cells *Saccharomyces cerevisiae*. MPhA reduced the frequency of direct mutations induced by UV radiation and nitric acid by 3.7 and 33 times, respectively. The frequency of spontaneous mutations (CANR) decreased by 6.4 times and mutations in ADE4–ADE8 loci decreased by more than 100 times. The high antimutagenic effect of MPhA is probably associated with the activation of DNA repair systems.[58]

3.4.2 In vivo Studies (On Animals)

In a large-scale experiment involving almost 1,000 animals the preventive and therapeutic impacts of MPhA on CBA mice-males genetically predisposed to hepatocarcinomas provided a long-term effect of increasing the expression of leukocyte beta 2 integrins LFA-1 and Mac-1 on peripheral blood cells involved in contact interactions of immune effectors with target cells. At the same time a decrease in the concentration of IL-6 and IL-10 in the blood serum of animals was revealed. This leads to increased expression of beta 2 leukocyte integrins LFA-1 and Mac-1 - ligands of histo-nonspecific adhesion molecules ICAM-1,2 on tumor cells. In this way the regulation of heterotypic adhesive interactions that cause the attachment of immune effectors to tumor cells can be restored contributing to the elimination of the latter.[59,60]

Prevention of dopaminergic neurons loss in the brain was accompanied by a decrease in the level of tumor formation, an increase in lifespan and maintenance of somatic status with correction of the stress hormone corticosterone serum level. Consequently, it is possible to assume control of the hepatocarcinogenesis process with increased antitumor protection and weakening of stress mechanisms including the participation of peripheral dopamine high content justifying the properties of the latter known from the literature as a stress regulator, antitumor agent and geroprotector. At the same time peripheral dopamine may play a role in the maturation of cytotoxic lymphocytes contributing to their conjugation with tumor cells.[61,62]

As a result, a 30% decrease in the frequency of tumor formation as well as statistically significant reduced number and size of spontaneous hepatocarcinomas were shown. The average lifespan of mice increased roughly by 20% while maintaining body weight, motor (behavioral) activity and coat without signs of alopecia. With the chronobiological transfer of the mouse age to humans the mice of

the control group lived about 62 "human years", in the preventive mode group – about 70, in the therapeutic – about 75 years.[63]

The antimetastatic and immunomodulatory effects of MPhA were studied in male mice of the (CBAX57BL/6) F1 strain with Lewis lung carcinoma (LLC) transplanted subcutaneously. Experimental mice were injected intragastrically with 2.5 ml/kg of dry MPhA extract 0.3% aqueous solution. No metastases were found in macroscopic revision of internal organs in mice treated with MPhA (100% effect). They were detected in lung tissue only microscopically during histological examination in 43% of cases against 100% in the control. At the same time the survival rate in the experimental mice was 93% (13 out of 14 mice) while only 50% in the control. MPhA also contributed to both spontaneous and mitogen-induced proliferative activity of T-lymphocytes. Thus, the antimetastatic properties of MPhA were revealed which correlates with the immunomodulatory effect on the T-cell immunity and increased survival of animals.[64]

MPhA also showed radioprotective properties in mice and dogs under conditions of acute and prolonged gamma irradiation. The animals' lifespan increased by 80% (mice) and 40% (dogs) with the best peripheral blood parameters.[65,66]

In mice with MPTP-induced parkinsonian syndrome (the model of aging disease) antioxidant and neuroprotective activities of MPhA were shown. The MPhA prevented the development of hypokinesia and rigidity of animals, dopamine and its metabolites decreasing, suppressed the content of serotonin and malondialdehyde in the striatum of mice almost to normal values. A decrease in the activity of caspase 3 and the level of DNA fragmentation in the substantia nigra was also revealed which implies suppression of neuronal apoptosis death.[67]

3.4.3 Studies in the Clinic

Clinical studies of MPhA were conducted in patients with leukoplakia of the oral mucosa (an example of precancerous disease), advanced gastric cancer (an example of incurable stage IV cancer), benign prostatic hyperplasia (BPH, an example of age-related hormonal disbalance disease in men), Parkinson's disease (age-related neurodegenerative disease). These studies supplemented and confirmed the results of experimental researches.

So, in the clinic and in the experimental researches the immunomodulatory effect of MPhA was revealed that was seen in the normalization of the T- and B-cell immunity as well as in the increase of NK cells level and growth of IL-2 receptors expression.[68–71] At the same time the use of MPhA in Parkinson's disease led to the suppression of negative activation of immune system cells showing anti-inflammatory activity.[72]

The interferon-inducing effect of MPhA was also revealed. It was expressed in the normalization of immune and interferon status indicators as well as in the induction of its own interferon (IFN-γ) production by peripheral blood lymphocytes. After the course of MPhA treatment the number of patients whose blood cells responded with IFN production increased significantly not only in relation to IFN drugs (gammapheron, reaferon. realdiron, leukinferon, intron A, roferon A) but its inducers (ridostin, cycloferon, neovir, amixin) as well. It is very important that all patients showed sensitivity to MPhA in terms of the production of their own IFN when exposed to MPhA. Thus, the fact of the individual tolerance absence to MPhA was established which favorably distinguishes this multicomponent composition from individual phytoadaptogens.[70]

The high interferon-inducing activity of MPhA was detected using a biological method (delayed destruction of the diploid fibroblast culture monolayer after the introduction of a test virus - vesicular stomatitis virus or mouse encephalomyocarditis virus). The results obtained allow us to conclude that MPhA has antiviral activity.[68]

In addition, the antistress activity of MPhA was determined in these patients - a decrease in the level of the stress hormone cortisol. MPhA also showed high antioxidant activity – suppressed lipid peroxidation with a decrease in the level of malondialdehyde, activation of catalase and glutathione antiperoxide system.[69–71]

The adhesive properties of MPhA are also shown on the example of precancer - oral leukoplakia confirming experimental studies on high-cancer CBA mice. Under the influence of MPhA on the epithelial cells of the leukoplakia lesions an increase in the expression of ICAM-1 adhesion molecules (ligands of beta2 integrins on immune effectors) was shown, a growth in the expression of receptors mediating apoptosis (Fas APO1) was noted as well as a decrease in the expression of the protein-marker of immature epithelial cells (keratin 17). Since phytoadaptogens are inducers of differentiation it is likely that the effect of MPhA is associated with the restoration of the oral mucosa epithelial cells differentiation program with the ensuing consequences – a weakening of oral mucosa epithelium keratinization, an increase in the level of physiological cell death as well as the elimination of pathological cells in the leukoplakia lesions by immune effectors. Clinical efficacy of MPhA was equal to 73% that is twice higher than that of conventional treatment with Vitamin A preparations. Taking into account the revealed properties, the therapeutic effect of MPhA against precancerous (leukoplakia) and the preventive effect in high-cancer mice MPhA can be considered to be a medication for cancer prevention.[69,73,74,75]

The use of MPhA in advanced gastric cancer was successful confirming the 100% antimetastatic effect obtained *in vivo* experiments on Lewis lung carcinoma. In patients who received only surgical treatment with MPhA the lifespan increased from 6.3 to 15.1 months. The

combination of MPhA and surgery with chemotherapeutic drugs increased the patients' lifespan from 5.4 to 14.3 months. At the same time in the groups using MPhA tumor markers carbohydrate antigen CA 19–9 and CEA levels were reduced. In addition, MPhA administration with conventional treatment of gastric cancer almost duplicated the number of polychemotherapy courses - 72 and 43 in the control. So, MPhA can be used by cancer patients with conventional treatment to slow down the tumor process and decrease the side effects of chemotherapy.[71]

As a result of MPhA use in patients with BPH a decrease (almost 3 times) in the age level of the chromosomal aberrations frequency in peripheral blood lymphocytes was also revealed. Administration of MPhA produced this parameter in lymphocytes of the patients lower than average population value in Russia and lower than that of the control groups without contacts with bad ecology as well as with industrial and household hazards. So, MPhA has proved itself as an adaptogen or geroprotector with an antimutagenic effect confirming *in vitro* studies.[76] The hormone-modulating effect of MPhA with BPH was also shown which was expressed in a testosterone level increase and an estradiol level decrease in the blood serum. At the same time MPhA normalized the level of prostate-specific antigen. MPhA, when inhibiting the development of age-related imbalance of testosterone and estradiol, interfered with the activity of the enzyme 5-α-reductase and together with the loss of age-related cortisol levels slowed down hyperplastic processes in the prostate, adrenaline release and also reduced the smooth muscle structures tone of the bladder base, posterior urethra and prostate gland. Thus, urination and, consequently, the quality of patients' life improved. So, consistent with the results obtained in high-cancer mice the effects in BPH improve the quality of patients' life contributing to slowing down the development of age-related stress reactions and demonstrating the geroprotective effect of MPhA.[77]

MPhA administration increased the efficacy of Parkinson's disease patients' conventional therapy inhibiting daily and motor activity violations as well as tremor, rigidity and bradykinesia according to UPDRS. Therefore, it was possible to taper gradually doses of L–DOPA-containing preparations that are very toxic and provide side effects. Stress hormone cortisol level was inhibited too. So, the quality of PD patients' life was improved. There are reasons to believe that taking into account *in vivo* studies MPhA has a neuroprotective effect restoring the dopamine-synthesizing function of reversibly damaged neurons and thus increasing the effectiveness of Parkinson's disease complex pathogenetic therapy.[74]

Thus, as a result of experimental and clinical studies it was found that MPhA has the properties of individual adaptogens (with the absence of a tolerance effect) including immuno- and hormone-modulating, antimutagenic, antistress, neuroprotective, antioxidant, radioprotective effects being a potential medication of preventive oncology, antitumor agent and geroprotector, showing a complex nature of action on the body.[78–81]

3.4.4 Comparison of in silico, in vitro, in vivo

The most of the antitumor and antimetastatic effects predicted in silico correspond to those found in vitro, in vivo and in clinic (hepatocarcinomas, lung and gastric cancer). In silico studies for more than 50 compounds demonstrated an antitumor effect against tumors of thirteen different localizations. Four possible mechanisms of antitumor activity have been identified including the antioxidant, apoptosis agonist, caspase 3 stimulant and NF kappa B transcription factor inhibitor, which are also consistent with the experimental results.

Taking into account the key role of the adhesive interactions' issues during the malignant growth, in silico analysis was performed on the effects of MPhA components in association with adhesive mechanisms. For antagonism of VLA (very late activation) beta 1 integrins molecules (providing heterotypic adhesion of tumor cells to other tissues promoting metastasis) the obtained positive Pa–Pi values do not exceed 0.25. For beta 2 integrins (providing heterotypic adhesion of tumor cells and cytotoxic lymphocytes) an agonistic effect was obtained at the highest values of Pa–Pi = 0.85. For VCAM (vascular cell adhesion molecules - promoting tumor angiogenesis) antagonism the obtained positive Pa–Pi values do not exceed 0.2. Respectively, in vivo and in clinic studies MPhA agonism in the expression of only beta 2 leukocyte integrins was shown.

Fifteen effects have been identified in silico that may increase the duration and improve the quality of patients' life: chemopreventive, antimutagenic, antioxidant, immunomodulatory and anti-inflammatory, hormone modulating, neuroprotective, antineurotic, radioprotective, antitoxic as well as effects associated with the therapy of Parkinson disease and its symptoms. All effects are estimated with a Pa–Pi value exceeded 0.4 for 35 or more phytocomponents. Effects associated with a positive action on the reproductive system of males and females were also revealed. For more than 20 MPhA phytocomponents with a threshold of Pa–Pi > 0.5 a therapeutic effect is rated in relation to BPH, male infertility, oligospermia, sexual dysfunction and improved fertility. The effects studied in vitro, in vivo and in clinic are rather similar.

Thus, the results of the computational estimation correspond to the data obtained for MPhA in vitro, in vivo and in clinical studies. The *in silico* analysis also showed that MPhA is a potential preventive and antitumor agent against many tumors and age-related diseases as well as a geroprotector showing a complex effect on the body. In addition, some other activities are predicted that may be the subject of future research - for example in relation to melanoma, breast, kidney and prostate cancer as well as anti-allergic and anti-coronavirus activities.

4 Conclusions

Multiphytoadaptogen is approved in Russia as a para-pharmaceutical agent and is consisted of 70 major components from 40 medicinal plants including *Panax ginseng*, *Rhodiola rosea, Eleutherococcus senticosus, Eucalyptus globules, Juniperus communis, Valeriana officinalis, Polygonum aviculare, Leonurus cardiac* etc. MPhA has been standardized by both analytical and biological methods. Pharmacological and toxicological characteristics of MPhA have been investigated *in vitro, in vivo* and in clinical studies. As a result of those investigations, a wide range of pharmacological effects (adaptogenic, immunomodulatory, antitumor, antimutagenic, neuroprotective, antistress etc.) exhibited by MPhA has been demonstrated.

Application of PASS and PharmaExpert software for *in silico* evaluation of biological activity spectra of separate phytocomponents and probable additive/synergistic effect of the whole composition revealed has demonstrated the reasonable correspondence between the predicted and known data on biological activities. For more than 50 MPhA phytocomponents antineoplastic effect is predicted against the thirteen tumors (Kaposhi' s sarcoma, brain cancer, breast cancer, endocrine cancer, head/neck cancer, liver cancer, lung cancer, osteosarcoma, pancreatic cancer, skin cancer, thyroid cancer, prostate cancer, renal cancer). Four probable mechanisms of action are revealed including antioxidant, apoptosis agonist, caspase 3 stimulant and transcription factor NF kappa B inhibitor, which also corresponds to the experimental results. Antimutagenic, immunomodulatory, radioprotective, neuroprotective and antiparkinsonian effects have been predicted for most phytocomponents as well. Effects associated with a positive action on the reproductive system of males and females were also revealed. Moreover, some previously not investigated biological activities have been predicted, which point to the most promising directions of further studies.

As a result of the interpretation of the prediction results, the secondary metabolites characterized by the most pronounced additive/synergistic effect were identified. Compounds with the probable antitumor effects include ginsenosides (Rb1, Rb2, Re, Rc, Rd, Rf, Rg1, Rg2, Ro) and aralosides (A, B, C). Compounds with the probable antineoplastic mechanisms of action include Quercetin, Luteolin and Naringetol. In terms of adhesion effects, Ethyl palmitate, Ethyl linoleate, Ethyl linolenate, Ethyl isovalerate show the greatest synergistic/adhesion effects. In terms of improvement of life quality and duration effects, the greatest additive/synergistic action was found for Rhodionin, Rosavin, Salidroside, Rosarin. The relevant data are presented in Table S7 of the Supporting Information.

Our investigations also demonstrate that computational methods of analysis can be considered to be an additional theoretical confirmation of the activity, justification and relevance of phytoadaptogenic pharmaceutical compositions.

Therefore, the PASS and PharmaExpert programs can be used to assess the pharmacological potential of multi-component complex pharmaceutical compositions containing a structural variety of natural products.

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Conflict of Interest

None declared.

Data Availability Statement

The data that supports the findings of this study are available in the supplementary material of this article

References

[1] P. M. Visscher, L. Yengo, N. J. Cox, N. R., *Science* **2021**, *373*, 1468–1473.
[2] I. Dagogo-Jack, A. T. Shaw, *Nat. Rev. Clin. Oncol.* **2018**, *15*, 81–94.
[3] J. J. Hornberg, in: *Designing Multi-Target Drugs*, (Eds: J. R. Morphy, C. J. Harris), Royal Society of Chemistry, Cambridge **2012**, pp. 1–13.
[4] R. Iyengar, *EMBO Rep.* **2013**, *14*, 1039–1042.
[5] C. Nogales, Z. M. Mamdouh, M. List, C. Kiel, A. I. Casas, H. H. H. W. Schmidt, *Trends Pharmacol. Sci.* **2022**, *43*, 136–150.
[6] A. S. Reddy, S. Zhang, *Expert Rev. Clin. Pharmacol.* **2013**, *6*, 41–47.
[7] R. Morphy, Z. Rankovic, *Curr. Pharm. Des.* **2009**, *15*, 587–600.
[8] D. J. Newman, G. M. Cragg, *J. Nat. Prod.* **2020**, *83*, 770–803.
[9] J. L. Medina-Franco, F. I. Saldívar-González, *Biomol. Eng.* **2020**, *10*, 1566.
[10] S. Stone, D. J. Newman, S. L. Colletti, D. S. Tan, *Nat. Prod. Rep.* **2022**, *39*, 20–32.
[11] Y. Zabolotna, P. Ertl, D. Horvath, F. Bonachera, G. Marcou, A. Varnek, *Mol. Inf.* **2021**, *40*, e2100068.
[12] D. J. Newman, *Curr. Ther. Res.* **2021**, *95*, 100645.
[13] A. Parveen, B. Parveen, R. Parveen, S. Ahmad, *J. Pharm. BioAllied Sci.* **2015**, *7*, 329–333.
[14] F. S. Li, J. K. Weng, *Nat. Plants* **2017**, *31*, 17109.
[15] A. G. Panossian, T. Efferth, A. N. Shikov, O. N. Pozharitskaya, K. Kuchta, P. K. Mukherjee, S. Banerjee, M. Heinrich, W. Wu, D. A. Guo, H. Wagner, *Med. Res. Rev.* **2021**, *41*, 630–703.
[16] O. A. Bocharova, Composition of ingredients for Phytomix-40. Patent RU 2099410 of 20/12/1997.
[17] O. A. Bocharova, Pharmaceutical composition presents antimutagenic activity. Patent RU 2477142 of 10/03/2013.

[18] O. A. Bocharova, A. Y. Baryshnikov, R. V. Karpova, E. V. Bocharov, V. A. Ilyenko, V. E. Shevchenko, O. P. Sheichenko, M. I. Davydov, A method for treatment of tumors in experimental animals with a high frequency of spontaneous tumor formation. Patent RU 2481849 of 20/05/2013.
[19] O. A. Bocharova, A. Y. Baryshnikov, R. V. Karpova, E. V. Bocharov, V. A. Ilyenko, V. E. Shevchenko, O. P. Sheichenko, M. I. Davydov, A method for preventing the development of tumours in experimental animals with a high frequency of spontaneous tumor formation. Patent RU 2507599 of 20/02/2014.
[20] O. A. Bocharova, O. N. Kurennaya, K. V. Kosikov, R. V. Serebryakova, N. B. Bodrova, G. N. Zatsepina, Method for biological control of adaptogens. Patent RU N° 2062300 of 20/06/1992.
[21] O. A. Bocharova, O. N. Kurennaya, R. V. Serebryakova, N. B. Bodrova, *Bio/Technology* **1993**, *8*, 28–34.
[22] O. P. Sheichenko, O. A. Bocharova, V. I. Sheichenko, E. V. Bocharov, V. A. Bykov, *Scientific works collection of VILAR* **2006**, *17*, 25–39.
[23] O. P. Sheychenko, O. A. Bocharova, V. I. Sheychenko, O. N. Tolkachev, E. V. Bocharov, R. V. Karpova, V. A. Bykov, *Vopr. Biol., Med. Farm. Khim.* **2007**, *5*, 20–25.
[24] O. P. Sheychenko, O. A. Bocharova, B. A. Krapivkin, E. V. Uyutova, O. N. Tolkachev, E. V. Bocharov, V. A. Bykov, *Vopr. Biol., Med. Farm. Khim.* **2008**, *6*, 18–28.
[25] O. P. Sheychenko, O. A. Bocharova, B. A. Krapivkin, E. V. Uyutova, R. V. Karpova, I. V. Kazeev, E. V. Bocharov, V. A. Bykov, *Vopr. Biol., Med. Farm. Khim.* **2012**, *10*, 52–59.
[26] I. V. Kazeev, O. A. Bocharova, V. E. Shevchenko, I. V. Kazeev, O. A. Bocharova, V. E. Shevchenko, R. V. Karpova, E. V. Bocharov, E. V. Uyutova, O. P. Sheichenko, V. G. Kucheryanu, M. A. Baryshnikova, *Theor. Found. Chem. Eng.* **2020**, *54*, 1242–1246.
[27] I. V. Kazeev, O. A. Bocharova, V. E. Shevchenko, R. V. Karpova, E. V. Bocharov, O. P. Sheichenko, V. G. Kucheryanu, M. A. Baryshnikova, V. B. Matveev, *Theor. Found. Chem. Eng.* **2021**, *55*, 1246–1257.
[28] O. A. Bocharova, I. V. Kazeev, V. E. Shevchenko, O. P. Sheichenko, V. V. Poroikov, E. V. Bocharov, R. V. Karpova, N. S. Ionov, V. G. Kucheryanu, V. S. Kosorukov, V. B. Matveev, I. S. Stilidi, *Pharm. Chem. J.* **2022**, *56(1)*, 32–38.
[29] O. A. Bocharova, V. E. Shevchenko, I. V. Kazeev, O. P. Sheychenko, N. S. Ionov, E. V. Bocharov, R. V. Karpova, A. A. Aksyonov, V. V. Poroikov, V. G. Kucheryanu, V. S. Kosorukov, *Pharm. Chem. J.* **2022**, *56(6)*, 29-37.
[30] O. A. Bocharova, R. V. Karpova, V. A. Golubeva, N. N. Kasatkina, O. N. Kurennaya, E. V. Bocharov, *Gig. Sanit.* **1999**, *5*, 60–63.
[31] O. A. Bocharova, R. V. Karpova, E. V. Bocharov, A. A. Vershinskaya, M. A. Baryshnikova, I. V. Kazeev, V. G. Kucheryanu, M. V. Kiselevskiy, *Ross. Bioterapevticheskii Zh.* **2020**, *19(2)*, 13–21.
[32] O. A. Bocharova, R. V. Karpova, E. V. Bocharov, A. A. Vershinskaya, M. A. Baryshnikova, I. V. Kazeev, V. G. Kucheryanu, M. V. Kiselevskiy, *Ross. Bioterapevticheskii Zh.* **2020**, *19(3)*, 12–20.
[33] S. Y. Pan, S. F. Zhou, S. H. Gao, Z. L. Yu, S. F. Zhang, M. K. Tang, J. N. Sun, D. L. Ma, Y. F. Han, W. F. Fong, K. M. Ko, *J. Evidence-Based Complementary Altern. Med.* **2013**, *2013*, 627375.
[34] J. Gertsch, *Planta Med.* **2011**, *77*, 1086–1098.
[35] A. A. Lagunin, R. K. Goel, D. Y. Gawande, P. Pahwa, T. A. Gloriozova, A. V. Dmitriev, S. M. Ivanov, A. V. Rudik, V. I. Konova, P. V. Pogodin, D. S. Druzhilovskiy, V. V. Poroikov, *Nat. Prod. Rep.* **2014**, *31*, 1585–1611.
[36] Y. Chen, J. Kirchmair, *Mol. Inf.* **2020**, *39*, e2000171.
[37] F. Pereira, *Mol. Inf.* **2021**, 40, e2060034.
[38] Y. V. Burov, V. V. Poroikov, L. V. Korolchenko, *Bull. Natl. Center Biol. Act. Comp.* **1990**, *1*, 4–25.
[39] A. Lagunin, A. Stepanchikova, D. Filimonov, V. Poroikov, *Bioinformatics* **2000**, *16*, 747–748.
[40] D. A. Filimonov, A. A. Lagunin, T. A. Gloriozova, A. V. Rudik, D. S. Druzhilovskiy, P. V. Pogodin, V. V. Poroikov, *Chem. Heterocycl. Compd.* **2014**, *50*, 444–457.
[41] D. A. Filimonov, D. S. Druzhilovskiy, A. A. Lagunin, T. A. Gloriozova, A. V. Rudik, A. V. Dmitriev, P. V. Pogodin, V. V. Poroikov, *Biomed. Chem. Res. Meth.* **2018**, *1*, e00004.
[42] C. Di Giorgio, F. Delmas, E. Ollivier, R. Elias, G. Balansard, P. Timon-David, *Exp. Parasitol.* **2004**, *106*, 67–74.
[43] B. Corominas-Faja, E. Santangelo, E. Cuyàs, V. Micol, J. Joven, X. Ariza, A. Segura-Carretero, J. García, J. A. Menendez, *Aging* **2014**, *6*, 731–741.
[44] N. N. Nassar, M. Y. Al-Shorbagy, H. H. Arab, D. M. Abdallah, *Neuropharmacology* **2015**, *89*, 308–317.
[45] F. M. Siraj, N. SathishKumar, Y. J. Kim, S. Y. Kim, D. C. Yang, *J. Enzyme Inhib. Med. Chem.* **2015**, *30*, 9–14.
[46] D. Y. Gawande, R. K. Goel, *J. Ethnopharmacol.* **2015**, *175*, 324–334.
[47] M. Alam, A. Khan, A. Wadood, A. Khan, S. Bashir, A. Aman, A. K. Jan, A. Rauf, B. Ahmad, A. R. Khan, U. Farooq, *Front. Pharmacol.* **2016**, *7*, 26.
[48] P. Pahwa, R. K. Goel, *Chem.-Biol. Interact.* **2016**, *260*, 208–218.
[49] A. Lagunin, M. Povydysh, D. Ivkin, V. Luzhanin, M. Krasnova, S. Okovityi, A. Nosov, M. Titova, S. Tomilova, D. Filimonov, V. Poroikov, *Mol. Inf.* **2020**, *39*, 2000093.
[50] L. H. Mervin, A. M. Afzal, G. Drakakis, R. Lewis, O. Engkvist, A. Bender, *J. Cheminf.* **2015**, *7*, 51.
[51] A. G. Malenkov, O. A. Bocharova, E. A. Modyanova, *Bull. of Discov. and Invent. Of USSR* **1987**, *42*, 3.
[52] O. A. Bocharova, *Issue RAMS.* **2002**, *1*, 22–25.
[53] H. Harjunpää, M. Llort Asens, C. Guenther, S. C. Fagerholm, *Front. Immunol.* **2019**, *10*, 1078.
[54] E. K. Kim, J. M. Ha, Y. W. Kim, S. Y. Jin, H. K. Ha, S. S. Bae, *FEBS Lett.* **2014**, *588*, 2971–2977.
[55] O. A. Bocharova, O. N. Kurennaya, K. V. Kosikov, R. V. Serebryakova, N. B. Bodrova, G. N. Zatsepina, Method for biological control of adaptogens. Patent (RU). 2062300, **1996**.
[56] O. A. Bocharova, M. A. Lizhenkova, O. N. Kurennaya, V. A. Knyazhev, *Bull. Exp. Biol. Med.* **2003**, *136*, 6, 615–617.
[57] O.A. Bocharova, R. V. Karpova, N. N. Kasatkina, L. G. Polunina, T. S. Komarova, M. A. Lyzhenkova, *Farm. Vestn.* **1999**, *50*, 380.
[58] O. N. Kurennaya, R. V. Karpova, O. A. Bocharova, I. V. Kazeev, E. V. Bocharov, *Russ. J. Genet.* **2013**, *49(12)*, 11190–1194.
[59] O. A. Bocharova, E. V. Bocharov, R. V. Karpova, V. A. Ilyenko, I. V. Kazeev, A. Yu Baryshnikov, *Bull. Exp. Biol. Med.* . **2014**, *157(2)*, 258–260.
[60] O. A. Bocharova, R. V. Karpova, E. V. Bocharov, A. A. Aksyonov, V. G. Kucheryanu, V. B. Matveev, *Lab. Anim.* **2021**, *4*.
[61] E. V. Bocharov, O. A. Bocharova, Y. N. Solov'ev, R. V. Karpova, *Bull. Exp. Biol. Med.* **2016**, *161(5)*, 727–730.
[62] O. A. Bocharova, A. V. Revishin, E. V. Bocharov, R. V. Karpova, V. G. Kucheryan, M. V. Utkina, V. B. Matveev, G. V. Pavlova, *Lab. Anim.* **2021**, *2*.
[63] E. V. Bocharov, R. V. Karpova, O. A. Bocharova, V. G. Kucheryanu, Z. S. Shprakh, *Bull. Exp. Biol. Med.* **2017**, *163(6)*, 789–792.
[64] O. A. Bocharova, R. V. Karpova, V. A. Golubeva, T. G. Filippova, N. N. Kasatkina, A. Yu Baryshnikov, *Bull. Exp. Biol. Med.* **1999**, *128(10)*, 403–407.
[65] E. V. Bocharov, R. V. Karpova, I. V. Kazeev, V. G. Kucheryanu, O. A. Bocharova, *Patol. Fiziol. Eksp. Ter.* **2013**, *3*, 55–58.
[66] R. V. Karpova, E. V. Bocharov, I. V. Kazeev, V. G. Kucheryanu, O. A. Bocharova, *Patol. Fiziol. Eksp. Ter.* **2013**, *4*, 51–54.

[67] E. V. Bocharov, V. G. Kucheryanu, G. N. Kryzhanovsky, O. A. Bocharova, V. S. Kudrin, S. A. Belorusceva, *Bull. Exp. Biol. Med.* **2006**, *141(5)*, 560–563.
[68] O. A. Bocharova, M. A. Lyzhenkova, M. V. Mezenceva, V. V. Semernina, V. A. Knyazhev, *Bull. Exp. Biol. Med.* **2003**, *136(6)*, 591–594.
[69] O. A. Bocharova, M. M. Pozharitskaya, T. L. Chekalina, M. V. Mezentseva, R. V. Karpova, V. V. Semernina, V. A. Knyazhev, *Immunologiya* **2004**, *25(1)*, 36–43.
[70] O. A. Bocharova, R. V. Karpova, V. B. Matveev, A. A. Aksyonov, M. A. Lyzhenkova, F. I. Ershov, M. V. Mezentseva, V. V. Semernina, *Ross. Bioterapevticheskii Zh* **2004**, *3(1)*, 90–95.
[71] O. A. Bocharova, M. I. Davydov, A. A. Klimenkov, A. Yu Baryshnikov, R. V. Karpova, S. V. Chulkova, E. G. Gorozhanskaya, V. A. Ilyenko, *Bull. Exp. Biol. Med.* **2009**, *148(1)*, 82–85.
[72] E. V. Bocharov, I. F. Ivanova-Smolenskaya, V. V. Poleshchuk, V. G. Kucheryanu, V. A. Il'enko, *Bull. Exp. Biol. Med.* **2010**, *149(6)*, 682–684.
[73] O. A. Bocharova, M. M. Pozharitskaya, T. L. Chekalina, M. A. Lyzhenkova, R. V. Karpova, M. V. Mezentseva, F. I. Ershov, *Bull. Exp. Biol. Med.* **2004**, *138(6)*, 578–583.
[74] O. A. Bocharova, T. L. Chekalina, M. M. Pozharitskaya, in: *Mechanisms of the pathogenesis of leukoplakia and the possibility of their correction with a complex phytoadaptogen. Exper. oncol. at the turn of the centure* (Eds: A. Y. Barishnikov, M. I. Davidov), Moscow **2003**, pp. 349–361.
[75] M. M. Pozgharickaya, O. A. Bocharova, T. L. Chekalina, V. F. Voronin, V. A. Knyazev, *Meth. Issue for Clin.Trials* **2004**, *M*, 46 p..
[76] N. P. Bochkov, O. A. Bocharova, A. A. Aksyonov, E. G. Gorozhanskaya, V. B. Matveev, R. V. Karpova, L. D. Katosova, N. V. Kosyakova, V. I. Platonova, A. N. Chebotarev, *Med. Genet.* **2005**, *4(1)*, 15–19.
[77] O. A. Bocharova, V. B. Matveev, R. V. Karpova, A. A. Aksyonov, E. G. Gorozhanskaya, A. N. Chebotaev, L. D. Katosova, V. I. Platonova, N. P. Bochkov, *Bull. Exp. Biol. Med.* . **2006**, *141(5)*, 616–619.
[78] O. A. Bocharova, M. I. Davidov, A. Y. Barishnikov, A. A. Klimenkov, V. B. Matveev, V. V. Pozharitskaya, I. A. Ivanova-Smolenskaya, R. V. Karpova, E. G. Gorozhanskaya, O. P. Sheichenko, B. P. Sukhanov, G. N. Kryzhanovsky, N. P. Bochkov, V. A. Bykov, V. A. Tutelyan, A. A. Vorobyov, V. A. Knyazhev, *Vestnik RAMS* **2009**, (*8*), 21–25.
[79] O. A. Bocharova, R. V. Karpova, E. V. Bocharov, A. A. Vershinskaya, M. A. Baryshnikova, I. V. Kazeev, V. G. Kucheryanu, M. V. Kiselevsky, V. B. Matveev, *Ross. Bioterapevticheskii Zh.* **2020**, *19(4)*, 35–44.
[80] O. A. Bocharova, V. B. Matveev, E. V. Bocharov, R. V. Karpova, V. G. Kucheryaunu, *Ross. Bioterapevticheskii Zh.* **2021**, *20(3)*, 17–24.
[81] O. A. Bocharova, V. B. Matveev, E. V. Bocharov, R. V. Karpova, V. G. Kucheryaunu, *Ross. Bioterapevticheskii Zh.* **2021**, *20(4)*, 42–50.

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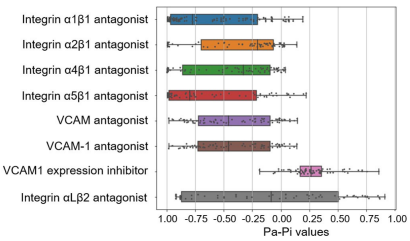

O. A. Bocharova, N. S. Ionov, I. V. Kazeev, V. E. Shevchenko, E. V. Bocharov, R. V. Karpova, O. P. Sheychenko, A. A. Aksyonov, S. V. Chulkova, V. G. Kucheryanu, A. V. Revishchin, G. V. Pavlova, V. S. Kosorukov, D. A. Filimonov, A. A. Lagunin, V. B. Matveev, N. V. Pyatigorskaya, I. S. Stilidi, V. V. Poroikov*