Study Of Ant aggregational and Anticoagulation Activity of The Five-Leaf Gynostemma (*Gynostemma Pentaphyllum* (Thunb.) Makino)

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Abstract. The article discusses the issues of studying the pharmacological activity of extracts of Gynostemma pentaphyllum: antiaggregational and anticoagulation activity.

Keyword: Gynostemma pentaphyllum, saponins, flavonoids, hyperoside.

Relevance. The search for new effective medicines is one of the main tasks of pharmacy. To solve this problem, it is necessary to study new types of medicinal plants and introduce them into medical practice. Correction of the general state of the body, namely, increased resistance, is possible when used as therapeutic and prophylactic agents - phytoadaptogens [1].
The main share of adaptogen plants is represented by endemic species (Far East, Altai Krai) [6]. In this regard, there is a question of expanding the range of medicines and introducing previously little-studied types of medicinal plant raw materials into practice by searching for new therapeutically effective sources. One of such promising crops adapted to the conditions of the Republic of Bashkortostan is the five-leaved gynostemma (Gynostemma pentaphyllum (Thunb.)) from the pumpkin family (Cucurbitaceae) (Figure 1).

Figure 1.*Gynostemma pentaphyllum*

Five-leaved gynostemma has versatile biological activity and is widely used in Southeastern medicine for the treatment and prevention of a wide range of diseases (Table 1).

Table 1 – Pharmacological effects of Gynostemma pentaphyllum

Pharmacological properties	Mechanisms of pharmacological action	Literature
Hypolipidemic	Gynostemma extract normalizes liver function and prevents liver obesity by modulating lipid metabolism via the PPAR-alpha-dependent pathway by inhibiting NF-kB factor, activates nuclear hormone receptors LXR, reduces oxidative stress, exerting a protective effect on hepatocytes in vitro, and lipid accumulation. The composition of gynostemma and arabinogalactan (1:2) leads to a significant decrease in the level of total lipids (by 33%), triglycerides (by 56%) and cholesterol (by 33%) in the liver and the level of triglycerides (by 56%) and cholesterol (by 28.4%) in the blood.	18, 19
Immunostimulating	Gynostemma extract increases the humoral and cellular immune response by activating T and B cells, stimulating the proliferation of lymphocytes, immunoglobulins (IgG1, IgG2), antibodies, cytokines in lymphocytes and acts as an immuno-supporting agent.	16, 17
Hypoglycemic	The gynostemma protects the beta cells of the pancreas, contributing to the preservation of the islets of Langerhans and the beta cells responsible for insulin secretion, which are disrupted during diabetes.Low doses of five-leaf gynostemma have a protective effect on the pancreas and contribute to the preservation of insulin secretion.	15, 19
Anti - ulcer	The extract of gynostemma promotes the restoration of affected cells, causes a noticeable result in the treatment of stomach ulcers and duodenal ulcers, showing gastroprotective properties.	22
Antiallergic	Five-leaf gynostemma effectively prevents the infiltration of immune cells and reduces hypersensitivity to allergens. The plant exhibits an antihistamine effect (inhibits eotaxin, a mediator of allergic diseases) and preserves the "open airways", inhibiting the narrowing of the airways, while not independently causing expansion.	9
Nootropic	Gynostemma extract regulates and protects the functions of the brain and nervous system. Having the ability to regulate the processes of excitation and inhibition in the central nervous system, it acts simultaneously as a calming and tonic, that is, it harmonizes the mental state (erases even the memory of stress) and as a result normalizes sleep, relieves pain.	10
Antioxidant	Г And this topic was studied on phagocytes using various models of oxidative stress, liver microsomes and vascular endothelial cells. The results of the studies showed a decrease in the content of superoxide anion and hydrogen peroxide in human neurophils and inhibition of chemiluminescent oxidative processes caused by zymosan	15, 23

	(glucan with repeating glucose units bound by β-1,3-glycoside bonds, used to experimentally cause inflammation) in human monocytes.Being a strong antioxidant, this plant prevents the development of the cancer process, promotes the restoration of cells already affected by cancer, stops the development of lung cancer, cervical cancer, liver, it also helps to eliminate harmful substances from the body: products of cancer intoxication, microbial and viral toxins, medicines and other pollutants, including radionuclides.	
Anti-inflammatory	The plant was tested on a model of eden caused by carrageenan. As a result of the analysis, the synthesis of nitric oxide and the activity of the iNOS enzyme of its synthesis were suppressed, the expression of antimicrobial proteins in the bladder was modulated, the activity of NF–kB and STAT3 factors in intestinal disease was inhibited, thereby exhibiting anti-inflammatory properties.	12
Antitumor	Gynostemma inhibits the growth of a solid H22 tumor (hepatocarcinoma), while an increase in the level of cytokines (IL-2, TNF-α, IFN-γ), the activity of natural killer cells and cytotoxic lymphocytes was observed.Gynostemma inhibited the growth of sarcoma tumors and Lewis lung cancer cells in vivo. It increases the content of a number of proteins associated with apoptosis of HSC-13 cells (oral cancer) and causes the death of ECA-109, TE-1 lines (esophageal cancer) due to the induction of lysosome proliferation.	23
Cytotoxic	The effect is associated with blocking cell division in the S, G0/G1 and G2/M phases, an increase in the content of reactive oxygen species (ROS) in the intercellular cells, an increase in the concentration of calcium ions in the intercellular cells and the production of pro-apoptotic proteins Bax, Bak and Bcl-X(L), a decrease in the synthesis of anti-apoptotic proteins Bcl-2 and Bad and depolarization of mitochondrial membranes.	8
Cardioprotective	The gynostemma has a vasodilating effect by stimulating the release of nitric oxide, inhibits the activity of the TF factor (tissue factor) associated with the development of atherosclerosis and other cardiovascular diseases. Gynostemma is an effective cardioprotector, acting on the estrogen receptors of blood vessels.	11
Bronchoprotective	The aqueous extract of gynostemma has a bronchodilating effect by inhibiting the expression of eotaxin in bronchial epithelial cells and histamine. In the ovalbumin-induced asthma model, the extract reduces respiratory tract inflammation, eosinophilia, and the expression of Otx2-related cytokines.	9
Hepatoprotective	On the model of ischemic-reperfusion liver damage, the gynostemma inhibits the proliferation of liver stellate cells that cause fibrosis, prevents liver fibrosis by inhibiting the differentiation of progepatocytes in myofibroblasts.	14

Despite studies by foreign authors of the biological properties and chemical composition of G. pentaphyllum, in the Russian Federation, the five-leaved gynostemma has not been practically studied. Therefore, it seems relevant to study the antiaggregational and anticoagulation activity of Gynostemma pentaphyllum.

The purpose of the study: to study the antiaggregational and anticoagulation activity of Gynostemma pentaphyllum (Thunb.) Makino.

Materials and methods of research. The object of research was: Gynostemma pentaphyllum (Thunb.) Makino grass grown in the conditions of the Republic of Bashkortostan (latitude (C) 54°49'4", longitude (C) 55°34'15"), as well as water-alcohol extracts of the herb. The aboveground part of the plant up to 2.5-3 m long was harvested during flowering and subjected to air-shade drying (Figure 1).

Figure 1. Raw Gynostemma pentaphyllum

To study the antiaggregational and anticoagulation activity, aqueous extracts were obtained from the studied plant raw materials, which were prepared in a ratio of 1:10, for this purpose the raw materials were crushed to a particle size of 3 mm. Decoctions were obtained from fruits, stems and seeds, infusions were prepared from shoots, herbs and leaves according to the method of GF IV edition [2.25]. Experiments to determine anticoagulation and antiaggregational activity in vitro were performed on the blood of healthy male donors aged 18-24 years. The total number of donors was 10 people. Blood sampling for the study of compounds in relation to the hemostasis system was carried out from the cubital vein using BD Vacutainer® vacuum blood sampling systems (Becton Dickinson and Company, USA). 3.8 % sodium citrate solution in the ratio of 9 was used as a stabilizer of venous blood:1. All tests were performed on platelet-rich and platelet-depleted plasma. Platelet-rich plasma samples were obtained by centrifugation of citrate blood at 1000 rpm for 10 minutes, platelet-free plasma at 3000 rpm for 20 minutes. The centrifuge OPN-3.02 (TNK DASTAN, Kyrgyzstan) was used in the work [7, 25,27]. The study of the effect on platelet aggregation was carried out using the Born method [Born G.G.V.Nature (London).-1962.-V.194.) on the aggregometer “AT-02” (NPF "Medtech", Russia]. Adenosine diphosphate (ADP) at a concentration of 20 micrograms/ml and collagen at a concentration of 5 mg/ml produced by Technologiya-Standart (Russia) were used as aggregation inducers. In the experiment, the maximum aggregation amplitude, aggregation rate, time to reach the maximum amplitude and disaggregation in the presence of the studied compounds during platelet aggregation were evaluated [28]. In collagen-induced platelet aggregation, the latency period during which phospholipase C is activated was evaluated (which leads to the formation of secondary mediators, resulting in the secretion of platelet granules and the synthesis of thromboxane A2).

Anticoagulation activity was determined by generally recognized clotting tests on an optical two-channel automated blood coagulation analyzer ASKa 2-01-"Astra" (SPC "Astra", Russia). The parameters of activated partial thromboplastin time (APTT), prothrombin time (PV) and fibrinogen concentration

according to A.Clauss were studied. Reagents produced by “Technology-Standard" (Barnaul, Russia) were used in the work.

To assess the pharmacological activity, the studied aqueous extracts were introduced into the blood plasma at the rate of 5% of the volume of the reaction mixture. The following comparison drugs were used: acetyloxybenzoic acid (Acetylsalicylic acid, Shandong Xinhua Pharmaceutical Co., Ltd., China) at a concentration of 2×10-3 M/l; Sodium Heparin (Sintez OJSC, Russia) at a concentration of 5×10-4 g/ml.

Reagent kits used for work at the in vitro stage were used for research: coagulation test kits manufactured by “Technology-Standard” (Barnaul): Tech-APTV-El-test, Tech-Fibrinogen-test, Tech-Plate-test (R); platelet aggregation inductors manufactured by “Technology-Standard" (Barnaul Barnaul): ADP, collagen.

Results and discussion.

According to the obtained data, the studies of the extraction of the gynostemma showed only a tendency to anticoagulation activity (prevent the formation of blood clots), comparable in effect to the reference drug - heparin solution. The data on the study of the effect of extracts of the herb gynostemma on platelet aggregation showed that the object reduces the maximum amplitude (degree of aggregation) and this indicates an effect on platelet aggregation, even slightly exceeds the indicators of the comparison drug - acetylsalicylic acid.

The indicators of the antiaggregational activity of Gynostemma pentaphyllum and the comparison drug, which was acetylsalicylic acid, are presented in Table 2.

Table 2 - Effect of Gynostemma pentaphyllum on platelet aggregation, Iu (0.25-0.75)

Code	Latency period, % of control	Maximum amplitude, % to control	Aggregation rate, % to control	Time to reach MA, % to control	Disaggregation, % to control
Grass GP	+4,6 (3,1-6,2)#	-14,4 (11,3-16,7)*,	-10,4 (8,3-12,1)*,	+18,6 (14,9-21,3)*,#	0,0 (0,0-0,0)††
Acetylsalicylic acid	-2,1 (1,1-2,6)	-13,7 (10,8-16,4)*,	-10,5 (7,6-12,3)*,	+10,5 (8,7-13,4)*,	0,0 (0,0-0,0)††

Note: The latency period is presented for collagen-induced platelet aggregation, the remaining parameters for ADP-induced platelet aggregation. *p≤0.05, **p≤0.001 - in comparison with the control; #p≤0.05, ##p≤0.001 - in comparison with acetylsalicylic acid. n=4. ††

Conclusions. Thus, taking into account the anticoagulation activity of the raw materials, the gynostemmas can be used in pathological processes associated with increased blood aggregation.

References

[1] Adaptogens and related groups of drugs - 50 years of searches / E.P. Studentsov, S.M. Ramsh, N.G. Kazurova [et al.] // Reviews on clinical pharmacology and drug therapy. - 2013. – Vol. 11, No. 4. – pp. 3-43.

[2] State Pharmacopoeia of the Russian Federation XIV ed. [Electronic resource] // Federal Electronic Medical Library, 2018. – Access mode: http://www.femb.ru/

[3] Ibragimova, E. F., & Yuldashev, S. J. (2012). The effect of diclofenac sodium on the exudative phase of inflammation in rats with hypotrophy. Postgraduate doctor, 52(3.1), 156-160.

[4] Kurkin, V.A. Determination of saponins in ginseng roots / V.A. Kurkin, A.S. Akushskaya // Pharmacy. - 2012. – No. 3. – pp. 18-20.

[5] Mironov, A.N. Guidelines for conducting preclinical studies of medicines / A.N. Mironov, N.D. Bunatyan. – Tula: Vulture and K, 2012. – 944 p.

[6] Pat. SU 1 507 394 A1 USSR, IPC A 61 K 35/78. Method of quantitative determination of flavonoids in plant raw materials / V.V. Belikov, N.T. Kolesnik; applicant and patent holder All-

Union Research Institute of Chemistry and Technology of Medicines – No. 4301201/28-14; application 25.08.1987; publ. 15.09.1989, Bul.

[7] No. 34.7. Plant Resources of Russia: Wild–growing flowering plants, their component composition and biological activity / A.L. Budantsev, L.M. Belenovskaya, N.A. Medvedeva [et al.]. - St. Petersburg.; Moscow: Association of Scientific Publications of the CMC, 2010. – Vol. 3. – 560 p.

[8] Guidelines for conducting preclinical studies of medicines. Part one. - M.: Vulture and K, 2012. - 944 p.

Aslam, I., Jiyanboyevich, Y. S., Ergashboevna, A. Z., Farmanovna, I. E., & Rajabboevna, A. R. (2021). Novel oral anticoagulants for treatment of deep venous thrombosis and pulmonary embolism. *Eurasian Research Bulletin*, *1*(1), 59-72.

[9] A novel *Gynostemma pentaphyllum* saponin and its adipogenesis inhibitory effect through modulating Wnt/beta-catenin pathway and cell cycle in mitotic clonal expansion / J. Liu, P. Yang, H. Shi [et al.] // Functional foods. – 2015. – Vol. 17. – P. 552-562.

[10] Bronchodilatory effects of the aqueous extract of *Gynostemma pentaphyllum* and gypenosides III and VIII in anaesthetized guinea-pigs / C. Circosta, R. De Pasquale, D. R Palumbo, F. Occhiuto // Pharmacy and pharmacology. – 2005. – Vol. 57, № 8. – P. 1053-1058.

[11] Chemistry and pharmacology of *Gynostemma pentaphyllum* / V. Razmovski-Naumovski, T.H. Huang, V.H. Tran [et al.] // Phytochemistry Reviews. – 2005. – Vol. 4, № 3. – P. 197-219.

Circosta, C. Cardiovascular effects of the aqueous extract of *Gynostemma pentaphyllum* Makino / C. Circosta, R. De Pasquale, F. Occhiuto // Phytomedicine: international journal of phytotherapy and phytopharmacology. – 2005. – Vol. 12, № 9. – P. 638-643.

[12] Evaluation of the anti-inflammatory and liver-protective effects of *Anoectochilus formosanus, Ganoderma lucidum* and *Gynostemma pentaphyllum* in rats / J.M. Lin, C.C. Lin, H.F. Chiu [et al.] // The American journal of Chinese medicine. – 1993. – Vol. 21, № 1. – P. 59-69.

[13] Goth, L. A simple method for determination of serum catalase activity and revision of reference range / L. Goth // Clinics chimica acta. – 1991. – Vol. 196, № 2-3. – P. 143-152.

[14] Gypenosides ameliorate carbon tetrachloride-induced liver fibrosis by inhibiting the differentiation of hepatic progenitor cells into myofibroblasts / J. Chen, X. Li, Y. Hu [et al.] // The American journal of Chinese medicine. – 2017. – Vol. 45, № 5. – P. 1061-1074.

[15] Hypoglycemic effect of *Gynostemma pentaphyllum* saponins by enhancing the Nrf2 signaling pathway in STZ-inducing diabetic rats / D. Gao, M. Zhao, X. Qi [et al.] // Archivers of pharmacal research. – 2016. – Vol. 39, № 2. – P. 221-230.

[16] Immunomodulatory and antioxidant effects of polysaccharides from *Gynostemma pentaphyllum* Makino in immunosuppressed mice / X. Shang, Y. Chao, Y. Zhang [et al.] // Molecules. – 2016. – Vol. 21, № 8. – P. 1085.

[17] Immunomodulatory effects of *Gynostemma pentaphyllum* Makino on human immune cells / B. Sriwanthana, W. Threesangsri, W. Wanavichet [et al.] // Traditional Medicine and Nutraceuticals. – 2005. – Vol. 680, № 25. – P. 165-169.

[18] Lipid-lowering, hepatoprotective, and atheroprotective effects of the mixture Hong-Qu and gypenosides in hyperlipidemia with NAFLD rats / S. Gou, H.-F. Huang, X.-Y. Chen [et al.] // Chinese Medical Association. – 2016. – Vol. 79, № 3. – P. 111-121.

[19] Megalli, S. Anti-hyperlipidemic and hypoglycemic effects of *Gynostemma pentaphyllum* in the zucker fatty rat / S. Megalli, N.M. Davies, B.D. Roufogalis // Pharmacy and pharmaceutical sciences. – 2006. – Vol. 9. – P. 281-291.

[20] Neuroprotective effects of the polyphenolic antioxidant agent, Curcumin, against homocysteine-induced cognitive impairment and oxidative stress / A. Ataie, M. Sabetkasaei, A. Haghparast [et al.] // Pharmacology, biochemistry and behavior. – 2010. – Vol. 96, № 4. – P. 378-385.

[21] Nizamova, A.A. The Determination of Antioxidant Activity of Ethanol Extracts of Gynostemma Pentaphyllum / Nizamova A.A., Galiakhmetova E.H. , Kudashkina N.V. [et al.] // Iranian Journal of Pharmaceutical Sciences, Vol 17 (1), 2021. – P. 91-98.

[22] Phytopreventative effects of *Gynostemma pentaphyllum* against acute indomethacin-induced gastrointestinal and renal toxicity in rats / C. Hesse, V. Razmovski-Naumovski, C. C Duke [et al.] // Phytotherapy research. – 2007. – Vol. 11, № 6. – P. 523-530.

[23] Synthesis and Antiaggregant Activity of 2-[3-Methyl-1-Ethylxanthinyl-8-Thio]Acetic Acid Salts Containing a Thietane Ring Khaliullin, F.A., Shabalina, Y.V., Samorodov, A.V., (...), Timirkhanova, G.A., Murataev, D.Z. 2018 Pharmaceutical Chemistry Journal. 52(1), c. 52-56

[24] Synthesis and diverse biological activity profile of triethyl-ammonium isatin-3-hydrazones. Bogdanov, A., Tsivileva, O., Voloshina, A., (...), Samorodov, A., Pavlov, V. 2022 ADMET and DMPK. 10(2), c. 163-179

[25] Synthesis, Antiaggregant, and Antioxidant Activity of 2-[1-iso-butyl-3-methyl-7-(thietanyl-3)xanthin-8-yl]thioacetic Acid Salts Khaliullin, F.A., Mamatov, Z.K., Timirkhanova, G.A., Samorodov, A.V., Bashirova, L.I. 2020 Pharmaceutical Chemistry Journal. 54(9), c. 891-896

[26] The hemostatic activity of bis (2-aminoethan-1-sulfonate) calcium. Gurevich, K.G., Urakov, A.L., Bashirova, L.I., (...), Gilmutdinova, A.S., Bondareva, N.A. 2018 Asian Journal of Pharmaceutical and Clinical Research. 11(11), c. 452-455

[27] Wang, T-X. Bioassay-guided isolation and identification of anticancer and antioxidant compounds from *Gynostemma pentaphyllum* (Thunb.) Makino / T-X. Wang, M-M. Shi, J-G. Jiang // RSC Advances. – 2018. – Vol. 8, № 21. – P. 23181-23190.