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Pharmacological Activity of Pinostrobin and Solid Dispersion Based on It

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Abstract

BACKGROUND: The low solubility of pinostrobin in water limits its use, causing technological difficulties and significantly reducing bioavailability. The "solid dispersion method" can significantly increase both the solubility and the release of a number of active substances from various dosage forms.

AIM: The purpose of this study is the pharmacological study of pinostrobin and solid dispersion based on it.

MATERIAL AND METHODS: Experiments were performed *in vitro* and *in vivo* on cells of human hepatocellular carcinoma (HepG2) and on 90 white outbred male and female rats and 24 outbred male mice. Methods were used to assess toxicity and determine antioxidants, hepatoprotective, capillary-strengthening, anti-inflammatory, and anti-ulcer activities. The effects were investigated by cytotoxicity, antioxidant, hepatoprotective, capillary-strengthening, anti-inflammatory, and antiulcer activity of the flavonoid was determined by pinostrobin and its solid dispersion, isolated from the buds of balsam poplar (*Populus balsamifera L.*).

RESULTS: The results of studying biological activity show that this compound is a promising substance for the prevention and complex therapy of various diseases.

CONCLUSION: It should be noted that pinostrobin can be considered a potential candidate for the targeted synthesis of a solid dispersion with more pronounced pharmacological effects and relatively high bioavailability.

Introduction

Recently, the class of natural flavonoids has been interesting among the promising pharmacologically active compounds. Currently, a wide range of pharmacological activities of bioflavonoids is known, including antioxidant, immunostimulating, antitumor, cardio-, radio-, antiallergic, and antiviral [1], [2], [3].

A significant number of natural antioxidants of the phenolic class present in medicinal herbs determines their antioxidant and anti-inflammatory effects [4]. The most important property of many phenolic compounds is their participation in redox reactions and in the processes of neutralisation of reactive oxygen species. According to their mechanism of action, flavonoids can be classified as antioxidants, chain-terminating substances whose molecules are more reactive than their radicals. They easily give up their electrons, turning the radical with which they reacted into a molecular product, while they themselves turn into a weak phenoxyl radical, which cannot participate in the continuation and chain reaction. Flavonoid-containing plants are the only raw materials used to produce P-vitamin preparations with antioxidant properties. P-active substances are represented by flavonols (rutin, quercetin, isoquercetin), anthocyanins, leukoanthocyanins and catechins [5].

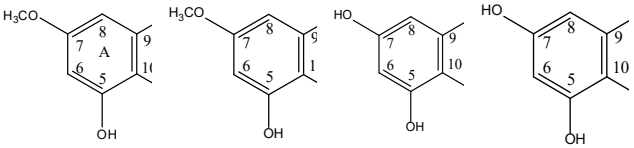
One of the widely represented flavonoids in plants is pinostrobin, which is a member of 22 families. This flavonoid molecule is one of the most hydroxylated and has a wide range of therapeutic effects.

Pinostrobin (1), a flavonoid discovered over 60 years ago from the heartwood of pine trees (Pinus strobus), attracts the attention of researchers due to its

practical availability from natural sources, a wide range of pharmacological activity and potential as a renewable chemical material for the synthesis of new biologically active compounds [6], [7], [8], [9], [10], [11], [12], [13], [14], [15].

Pinostrobin (1) has a free proton at carbon atoms C-3, C-6 and C-8, as evidenced by the electrical dipole moment of the pinostrobin molecule, which is μ=2.1581D and reacts with reactive oxygen species and other free radicals, neutralising them and preventing oxidative damage to cells.

One of the main sources of pinostrobin (1) is the buds of the balsam poplar (Populus balsamifera L.), the yield of which is 2.4% based on air-dry raw materials. In addition to pinostrobin (1), balsam poplar buds contain flavonoids: tectochrysin (2), pinocembrin (3), and chrysin (4) [16].



(1) (2) (3) (4)

Based on the results of computer modeling of the biological activity of pinostrobin (1) using the PASS computer system (Prediction of Activity Spectra for Substances) relatively probable types of pharmacological activity are membrane stabilizing, antioxidant, angioprotective, anti-inflammatory, hepatoprotective and antiviral.

Despite its low solubility in water, pinostrobin (1) exhibits potential pharmacological activity [8, 17]. Based on the results of pharmacokinetic studies, it was found that pinostrobin (1) is practically insoluble in water, and the addition of surfactants slightly increases the solubility of the substance, achieving an absolute bioavailability of pinostrobin (1) of 11%.

Therefore, an urgent task is the synthesis of a water-soluble form of pinostrobin (1) and the study of the pharmacological activity of the original flavonoid, and the water-soluble solid dispersion synthesised on its basis, determining its effectiveness and bioavailability.

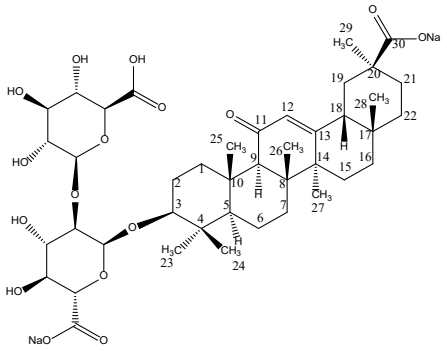
The purpose of this study is a pharmacological study of pinostrobin and a solid dispersion based on it.

Materials and Methods

Object of study

Pinostrobin (5-hydroxy-7-methoxy-2-phenylchroman-4-one) (1), isolated from the carbonic acid extract of Populus balsamifera L., a crystalline substance of composition C16H14O4, with a melting point of 96-99°C (ethyl acetate), [α]D20 – 57.50 (c 1.1; ethanol). Molecular weight 270.28 g/mol.

Disodium salt of glycyrrhizic acid (5) - a derivative of plant saponin (CFS, 98%), produced by Shaanxi Sciphar Biotechnology Co., Ltd (Xi'an, China), gross formula C42H60O16Na2, is a gray powder with mustard shade.



(5)

The synthesis of solid dispersion (SD) was carried out by the “simple mixing” method - the calculated amounts of the complexing agent and pinostrobin (1) were ground together for 1 min in a porcelain mortar until a homogeneous mixture [[17], 18].

A solid dispersion of pinostrobin with the disodium salt of glycyrrhizic acid, synthesised by the “simple mixing” method at a ratio of 1:2, is a powdery, opaque beige mass with a solid consistency. Physicochemical data, composition and structure of the synthesised solid dispersion are determined by microcrystalloscopy, X-ray phase analysis, and NMR relaxation.

Experimental animals

The experiments used intact male and female mice and rats. The animals were kept under standard laboratory conditions with free access to food and water. The Institutional Animal Care and Use Committee (IACUC) approved all experimental procedures. The conclusion of the Ethical Committee of the NJSC “Medical University of Karaganda” No. 9, Protocol No. 4, dated 12/06/2021, is included.

Molecular docking on biological targets

Three-dimensional receptor structures are used from the RCSB PDB database (http://www.rcsb.org/): endothelial NO-synthase – PDB ID: 4NOS; cystathionine-gamma-lyase – PDB ID: 3COG; phosphodiesterase 5 – PDB ID: 1UDT; angiotensin-converting enzyme – PDB ID: 6F9V; epoxide hydrolase – PDB ID: 4JNC; L-calcium channel – PDB ID: 3G43; β2-adrenergic receptor – PDB ID: 3SN6 [19].

Molecular docking was carried out using the Maestro graphical interface of the Schrödinger Suite software package. XP docking mode was used (extra precision). The final results were based on the value of the GScore evaluation function, which shows the binding energy of the ligand to the target molecule.

Ligand efficiency (LE) was calculated using the formula (-GScore)/HA, where GScore is the value of the calculated estimated binding energy, and HA is the number of heavy atoms in the ligand structure. Values ≥ 0.3 were taken as an acceptable level of ligand efficiency.

Cytotoxicity assessment

The effect of pinostrobin (1) and its solid dispersion with disodium glycyrrhizic acid on the viability of human hepatocellular carcinoma cells (HepG2) was assessed using a neutral red test at concentrations from 3.6 μM to 1000 μM.

HepG2 cells were cultured in a complete nutrient medium (CMM) (DMEM/F-12, 292 mg/L L-glutamine, 50 mg/L gentamicin, 10% FBS) for at least 3 passages. To assess the cytotoxic activity of the studied samples after transplantation of cells onto a T75 culture flask and the cells reaching 80-90% confluency, the medium was removed, the cells were washed with 5 ml of 1X Phosphate-Buffered Saline (PBS), 5 ml of Trypsin-EDTA was added to the culture flask, and the vial was placed in a CO2 incubator for 7 minutes. Next, 5 ml of CMM was added to the culture flask to neutralise trypsin. The supernatant was removed, and the cells were carefully resuspended in 1 ml of fresh CMM. A 10 μL aliquot was then removed to calculate the cell concentration in the suspension using a Countess II FL Automated Cell Counter and Cell Viability Analyzer. Cells in the amount of 10 thousand cells per well of the plate were distributed in 100 μl into 60 wells of a 96-well plate, all of the edge wells of which were filled with 200 μl of water.

The next day, the medium in the wells with cells was replaced with a fresh one. Aqueous solutions of the solid dispersion sample under study were added in the concentration range of 3.6 μM - 1000 μM. A sample of pinostrobin (1) was dissolved in dimethyl sulfoxide (DMSO) and added in a concentration range of 3.6 μM - 1000 μM to the appropriate plates. In this case, the final concentration of dimethyl sulfoxide (DMSO) in the wells of the plate was no more than 0.5% by volume. Plates with cells were placed in a CO2 incubator for 24 hours.

After incubation, the medium with substances was removed from the wells, the cells were washed once with 200 μl of 1X Phosphate-Buffered Saline (PBS), and 100 μl of neutral red CMM (40 μg/ml) was added to each well. The plates were placed in a thermostat for 2 hours at 37oC. The incubation medium with the dye was carefully removed, the cells were washed once with 200 μl of 1X Phosphate-Buffered Saline (PBS), and 150 μl of a mixture of 96% ethanol: deionised water: glacial acetic acid (50:49:1) was added to each well to extract the bound dye. The optical density was measured at a wavelength of 540 nm and a reference wavelength of 650 nm using a Tecan Infinite200 pro m plex multifunctional microplate reader [20].

Antioxidant activity

One of the decisive factors in enhancing the antioxidant effect of compounds is the presence of a phenoxyl fragment, which inhibits free radical processes. To determine the antioxidant activity of pinostrobin (1) and its solid dispersion, the method of initiated biochemiluminescence was used. The method is based on determining the iron-reducing ability of the test object, which was assessed spectrophotometrically on an Agilent Cary 60 instrument. A comparative assessment of the antioxidant activity of solutions of the studied compounds was carried out with respect to ascorbic and gallic acids.

To determine the iron-reducing potential of samples, add 0.1 ml of an alcohol solution to the test sample in the concentration range 0.25;0.5; 0.75; 1.0 mg/ml, 0.25 ml of phosphate buffer (0.2 M, pH6.6) and 0.25 ml of 1% solution of potassium hexacyanoferrate (III) are added. The reaction mixture is incubated for 20 minutes at a temperature of 50ºC; the reaction is stopped by adding 0.25 ml of 10% trichloroacetic acid solution. The mixture is centrifuged for 10 minutes (3000 rpm). The top layer of 0.5 ml is mixed with 0.5 ml of distilled water and 0.1 ml of 0.1% FeCL3. Optical density is measured at 700 nm on a Cary 60 spectrophotometer. AOA was compared with the activity of ascorbic and gallic acid [17] to evaluate AOA.

Hepatoprotective activity

Experiments to study hepatoprotective activity in vitro were conducted at non-toxic concentrations of the studied samples - 30 μM and 60 μM.

The effect of samples of pinostrobin (1) and its solid dispersion on intracellular lipid accumulation induced by 1 mM oleic acid and 0.5 mM palmitic acid in HepG2 cell culture was assessed by measuring the fluorescence of the lipophilic dye Nile red [21]. Since free fatty acids (FFA) induce oxidative stress, the effect of pinostrobin samples and its solid dispersion on the production of reactive oxygen species (ROS) in HepG2 cell culture was also studied using the intracellular probe 2,7-dichlorodihydrofluorescein diacetate (DCFDA) [22].

A medium with a high content of oleic (1 mM) and palmitic (0.5 mM) acids was prepared from the corresponding stock solutions (30 mM sodium salt of each fatty acid in 0.9% sodium chloride solution). Pre-stock solutions of fatty acids were heated on a solid-

state heater to 700 C until completely transparent. The prepared stock solutions were added to the DMEM/F-12 incubation medium containing 292 mg/L L-glutamine, 50 mg/L gentamicin and 2% BSA without fatty acids (300 μM) and placed on a rotator for mixing at 370 C within 20 minutes. Simultaneously, a control medium was prepared according to a similar scheme, excluding the addition of fatty acids.

The pinostrobin sample was dissolved in dimethyl sulfoxide (DMSO) and added to the appropriate wells of the plate to final concentrations of 30 μM or 60 μM. In this case, the final concentration of dimethyl sulfoxide (DMSO) in the wells of the plate was no more than 0.5% by volume. Plates with cells were placed in a CO2 incubator for 24 hours.

After incubation, the medium with substances was removed from the wells, and the cells were washed once with 200 μl of 1X Phosphate-Buffered Saline (PBS). To assess intracellular lipid accumulation, a working solution of the fluorescent dye Nile Red with a concentration of 1 μg/ml in 1X PBS was prepared from the stock solution (0.5 mg/ml in dimethyl sulfoxide (DMSO)). 150 μl of the working solution was added to the cells, and the plates were incubated for 15 minutes in the dark at room temperature, after which the cells were washed twice with 1X PBS, 100 μl of 1X PBS was added to the wells, and the fluorescence intensity was assessed at an excitation wavelength of 488 nm and an emission wavelength of 550 nm using a multifunctional microplate reader Tecan Infinite200 pro m plex. The results obtained were normalised to the amount of cellular DNA in the wells of the plates.

The amount of intracellular ROS was recorded after incubation of cells with the fluorescent probe DCFDA. A working reagent solution (10 μg/mL in 1X PBS) was prepared by diluting the stock solution (5 mg/mL in ethanol). 100 μl of the working solution was added to the wells, and the plates were incubated for 20 minutes in the dark at 370 C. Then the cells were washed with 1X PBS, and the fluorescence intensity was assessed at an excitation wavelength of 485 nm and an emission wavelength of 530 nm using a multifunctional microplate reader Tecan Infinite 200 pro m plex. The experiments were carried out in two independent series.

Capillary-strengthening activity

The study of the capillary-strengthening effect of pinostrobin (1) and its solid dispersion was carried out according to the method of P. Golikov [23]. The experiments were conducted on 30 white outbred male rats weighing 170-210 g. Under anaesthesia, the experimental animals were fixed on the operating table in a supine position. The femoral vein on the right hind limb was exposed, and a 1% solution of trypan blue was injected intravenously at a rate of 2 mg/kg. 10 minutes after intravenous administration of trypan blue, phlogogenic substances were injected intradermally into the shaved area of the abdomen. The following was used as a phlogogenic agent: 0.1% solution of zymosan, 1% histamine solution, egg white, and formalin 3%. The time of onset of staining of papules caused by the introduction of phlogogenic agents was taken into account. Pinostrobin and its solid dispersion and the reference drug diclofenac sodium (8 mg/kg) are administered intragastrically once a day for 3 days and on the 4th day, 1 hour before the administration of phlogogens.

Anti-inflammatory activity

The local anti-inflammatory activity of pinostrobin (1) and its solid dispersion with glycyrrhizic acid disodium salt was assessed by inducing an inflammatory response caused by 12-O-tetradecanoylphorbol-13-acetate (TPA).

The experiments were conducted on 24 white outbred male mice weighing 30-33 g. Six experimental groups and one control group of three mice each were formed. Initially, all groups of mice were treated with 20 μl of TPA at a concentration of 0.125 μg/μl, and the test substances were applied 30 min later. The substances were dissolved in a 50:50 volume ratio of acetone water and administered to groups of three mice in the right ear of each animal. Measurements were carried out as follows: for the first 6 hours, measurements were taken every 2 hours, and then after 24 and 48 hours using a Fowler Ultra-Cal Mark III digital micrometre (Massachusetts, USA). The test substances were dissolved in acetone and then injected locally in a volume of 20 μl into the right ear of each mouse. Three experimental groups received pinostrobin (1) in doses of 1.5, 2, and 3 mg; the other three experimental groups received a solid dispersion of pinostrobin in doses of 1.5, 2, and 3 mg. Animals in the control group were injected with water in a dose of 3 ml per ear.

Antiulcer activity

The antiulcer activity of pinostrobin (1) and its solid dispersion with the disodium salt of glycyrrhizic acid was studied in a model of acute ethanol ulcer in 60 white outbred male and female rats weighing 170-220 g. The model of acute gastric ulcer was reproduced using absolute ethanol. 24 hours before the experiment, the animals were deprived of food and water. Absolute ethanol was administered in a volume of 1 ml into the stomach 1 hour after administering a solid dispersion of pinostrobin at a dose of 150 mg/kg. Euthanasia of experimental and control animals was carried out on the 24th day from the start of the experiments. Then the stomachs were opened, the mucous membrane was washed with saline solution, and the nature and number of destructive lesions on the gastric mucosa were determined macroscopically using a magnifying glass.

Processing results

Statistical processing of the results was carried out using the program “GraphPad Prism v. 6.0" (GraphPad Software Inc., USA). The results obtained are presented as “average value ± standard error of the mean value”. Differences were considered significant at a significant level of $p<0.05$. The results are based on three to four independent experiments.

The obtained data were statistically processed using the Statistica 6.0 software package (USA). The data obtained during the experiments were processed statistically by generally accepted methods for a small sample, with the determination of the average value (M) and the standard error of the average (m) using the formula M ± m. The reliability of the results (P) was assessed using Student's t-test [24]. Differences were accepted as significant at $P < 0.05$.

Results and Discussion

Molecular docking on biological targets

Molecular docking of pinostrobin (1) was carried out with putative biological targets for vasodilator activity (endothelial NO synthase, cystathionine gamma-lyase, phosphodiesterase 5, angiotensin-converting enzyme, epoxide hydrolase, L-calcium channel and β2-adrenergic receptor).

The interaction of pinostrobin (1) with amino acid residues of the receptors is presented in Figures 1-4.

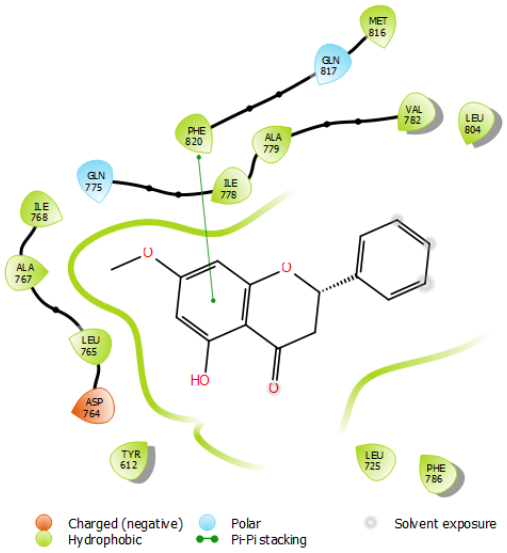


Figure 1: Interaction of phosphodiesterase 5 with pinostrobin

Through molecular docking, it was revealed that pinostrobin demonstrated the best binding energy values in complex with epoxy hydrolase (-9.652 kcal/mol) and phosphodiesterase 5 (8.779 kcal/mol).

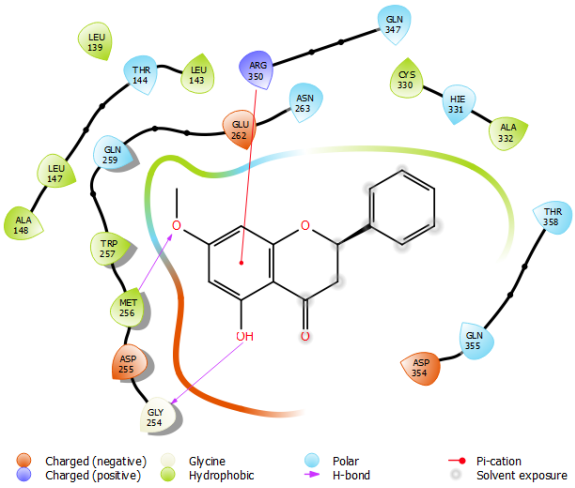


Figure 2: Interaction of angiotensin-converting enzyme 5 with pinostrobin

Pinostrobin showed relatively strong binding with the angiotensin-converting enzyme (-7.009 kcal/mol) and with the β2-adrenergic receptor (-7.174 kcal/mol).

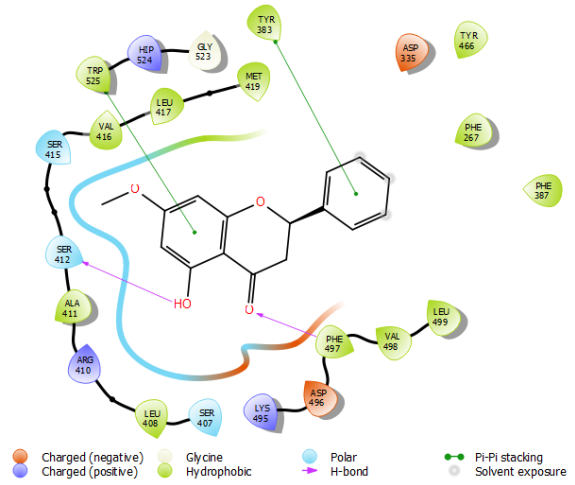


Figure 3: Interaction of epoxyhydrolase with pinostrobin

The best values of ligand efficiency were shown by pinostrobin in complex with epoxyhydrolase (LE equal to 0.48) and with phosphodiesterase 5 (LE equal to 0.44).

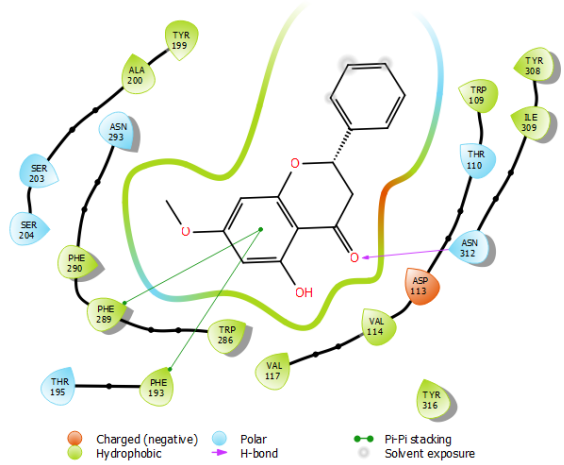


Figure 4: Interaction of β2-adrenergic receptor with pinostrobin

Pinostrobin also has relatively good ligand efficacy (LE ≥ 0.3) in combination with angiotensin-converting enzyme (LE 0.35) and with β2-adrenoreceptor (LE equals 0.36).

Cytotoxicity assessment

The cytotoxicity of pinostrobin (1) and its solid dispersion against human hepatocellular carcinoma cells (HepG2) was assessed in an experiment in vivo to select the dose to be studied when studying antioxidant, hepatoprotective, anti-inflammatory and antiulcer activities in the concentration range from 3.6 μM to 1000 μM.

The results of the assessment of the influence of the tested objects of pinostrobin (1) and its solid dispersion with the disodium salt of glycyrrhizic acid in a ratio of 1:2 on the viability of human hepatocellular carcinoma cells (HepG2) are presented in Figures 5-6.

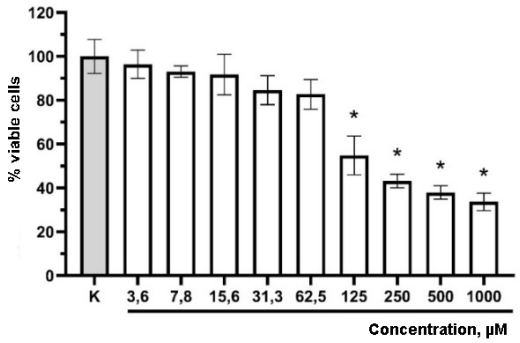


*Figure 5: Effect of pinostrobin substance on the viability of HepG2 cells in the neutral red test during incubation for 24 hours. Notes: in all cases n = 6; * – differences compared to the "Control" wells are statistically significant*

In the cytotoxicity assay, the IC50 values for pinostrobin (1) and its solid dispersion were 253.4 and 259.1 μM, respectively, which indicates their low activity in this experiment.

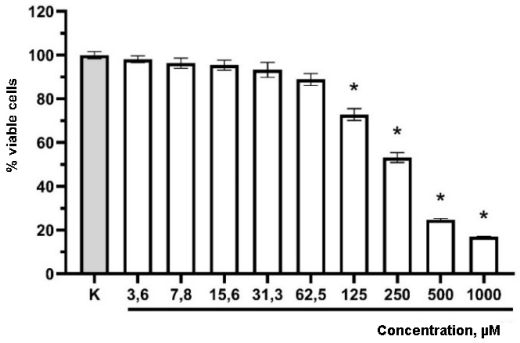


*Figure 6: Effect of pinostrobin solid dispersion substance on the viability of HepG2 cells in the neutral red test during 24 h incubation. Notes: in all cases n = 6; * – differences compared to the "Control" wells are statistically significant*

The results of our studies do not coincide with the data of other researchers who also studied the cytotoxicity of pinostrobin (1) [25]. When SK-BR-3, MCF-7 and PC3 cells were exposed to pinostrobin, IC50 values of 94.3 μM, 84.9 μM and 86.7 μM were obtained. Sukardiman et al. [26] studied its effect on cultured breast cancer cells and concluded that the anticancer effect of pinostrobin (1) is due to its inhibitory activity against topoisomerase I enzymes, which promote DNA replication. Extensive studies have shown that pinostrobin (1) also has apoptotic induction activity against T-47D human breast cancer cells through the p53 and bax pathways and also inhibits COX-2 expression in vitro.

The results of our experiments and the available literature data [25], [26] differ due to the different reactions or sensitivity of HepG2 cells to pinostrobin (1) and its solid dispersion, which requires additional research.

Antioxidant activity

Flavonoids are characterised by antioxidant activity, which is associated with the structure of their molecules, namely the presence of mobile protons in the aromatic system and the formation of a radical. The structural features of molecules allow flavonoids to easily transfer electrons to free radicals, which leads to the stabilisation and neutralisation of their destructive potential [17], [27].

Under the influence of reactive oxygen species, the structure of DNA molecules is disrupted. Antioxidants help stop the negative effects of oxidative stress on the cell, thereby being one of the factors in the implementation of genoprotective activity. Recently, much attention has been paid to the search for plant components that can neutralise the effects of pro-oxidants since substances of plant origin are usually less likely to cause adverse reactions and are often cost-effective. An important issue is elucidating the mechanism of action of the antioxidant activity of plant compounds. A description of methods for studying the antioxidant activity of pinostrobin (1) and its solid dispersion and their proposed mechanisms of action is provided.

The relevance of the problem of establishing the dependence of the antioxidant activity of individual compounds on their molecular structure is obvious. It is generally accepted that one of the decisive factors in enhancing the antioxidant effect of compounds is the presence of a phenoxyl fragment, which inhibits free radical processes. Therefore, we considered pinostrobin (1) and its solid dispersion with disodium glycyrrhizic acid as a potential antioxidant. We screened for antioxidant activity in vitro samples of pinostrobin and its solid dispersion with the disodium salt of glycyrrhizic acid (Table 1).

Table 1: Parameters of initiated chemiluminescence (ICL) of lipids in the presence of samples of pinostrobin and its derivatives

Substance	h, conventional units	τ, min	tgα	H, conventional units
Pinostrobin (1)	1.34 ± 0.11	3.04 ± 0.13	3.10 ± 0.29	6.12 ± 0.51
Pinostrobin solid dispersion	1.44 ± 0.12	2.90 ± 0.20	3.20 ± 0.29	6.40 ± 0.49
Ionol	2.17 ± 0.13	7.61 ± 0.15	2.69 ± 0.13	6.34 ± 0.51
Control	2.60 ± 0.10	2.08 ± 0.16	3.50 ± 0.29	7.10 ± 0.55

*All samples with titer T = 10 mg/ml in ethanol.

It was found that pinostrobin (1) at a concentration of 0.21 mM increases the latent period to 3.04 ± 0.13 minutes ($p<0.001$) in contrast to the control, for which the latent period was 2.08 ± 0.16 minutes (h=5). Increasing the concentration of pinostrobin (1) by 2 times adequately reduces the level of luminescence and lengthens the latent period of luminescence to 5.54±0.1 minutes ($p < 0.001$). A further increase in concentration leads to complete quenching of the glow, which is observed in the case of ionol with a concentration of 0.95 mM. This fact indicates that compound (1), in terms of antioxidant activity, is not inferior to the synthetic antioxidant ionol, which belongs to phenolic antioxidants.

Substance (1) exhibits an antioxidant effect at a concentration of 0.14 mM, which is 1.2 times higher than the control value. A further increase in the concentration of pinostrobin (1) leads to an increase in the latent period of ultra-weak luminescence, which indicates a pronounced antioxidant effect. Moreover, the increased activity of substance (1) is due to the presence of the second OH group in the aromatic structure.

The values of fast (h) and slow (H) flash characterise the level of peroxide processes and the state of the antioxidant defence of the system. According to Table 1, the antioxidant effect of samples of pinostrobin and its solid dispersion with disodium salt of glycyrrhizic acid is less than that of ionol.

When studying the biotransformation of pinostrobin (1) in an experiment on animals, it was found that the sample was excreted from the body of animals predominantly unchanged. The second most important way of eliminating pinostrobin solid dispersion is its elimination in the form of a glucuronic conjugate. A pharmacokinetic study of rat blood plasma after oral administration of pinostrobin at a dose of 0.5 mg/kg made it possible to determine the half-life (t1/2) of the flavonoid, which was 6.26 ± 0.31 hours.

Antioxidant ability pinostrobin (1) may be one of the mechanisms to inhibit enzyme activity, participating in inflammation and pain relief, such as prostaglandins, cyclooxygenase 1, and cyclooxygenase 2. This is explained by the anti-inflammatory and painkiller action of pinostrobin (1), which strengthened due to improvements in its solubility by using complex inclusion with disodium salt of glycyrrhizic acid.

The hepatoprotective effect of plant flavonoids is due to their antioxidant, membrane-stabilizing and stimulating properties that stimulate the reparative potential of liver cells. Therefore, our further research aimed to study the hepatoprotective activity of pinostrobin (1) and its solid dispersion.

Hepatoprotective activity

The hepatoprotective effect, to varying degrees, can be exhibited by various pharmacological agents that improve metabolic processes in the body, inhibit lipid peroxidation (LPO), have antihypoxic activity, protect mitochondrial and microsomal enzymes from damage, slow down collagen synthesis and increase collagenase activity. At the first stage of the experiment, LC50 was determined for the cell culture used. The effect of pinostobin (1) and its solid dispersion on cells in culture is assessed by their survival.

Based on the data obtained, experiments to study hepatoprotective activity in vitro were conducted at non-toxic concentrations of the studied samples – 30 μM and 60 μM.

Figures 7, 8, and Table 2 show the results of the assessment of the influence of the test objects of pinostrobin (1) and its solid dispersion on the accumulation of intracellular lipids in human hepatocellular carcinoma cells (HepG 2) when incubated in a medium containing 1 mM oleic acid and 0.5 mM palmitic acid.

As a result of the experiments, it was established that incubation of the HepG2 cell line in the presence of oleic (1 mM) and palmitic (0.5 mM) acids led to a significant increase in the level of intracellular lipids, as evidenced by an increase in the fluorescence of the lipophilic dye Nile Red by 6.1 times ($p <0.001$) and 4.0 times ($p <0.001$) in the first and second series of experiments, respectively.

To confirm the observed effect, visualisation was performed using a fluorescence microscope Leica DMi6 (Figure 7).

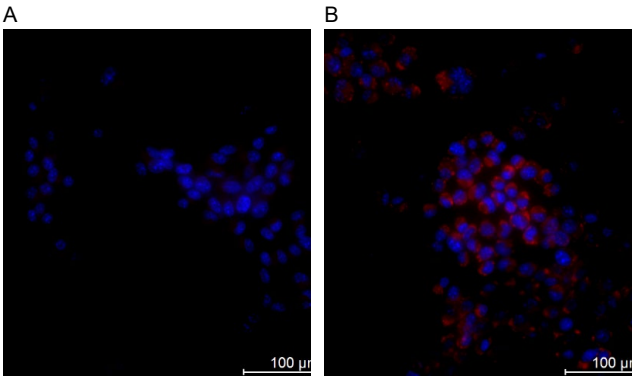


Figure 7: Effect of a medium with normal (A) and increased content of oleic (1 mM) and palmitic (0.5 mM) acids (B) on the fluorescence of the lipophilic dye Nile Red in HepG2 cells during incubation for 24 hours

Table 2: Fluorescence intensity ratio of the lipophilic dye Nile Red to the fluorescence intensity of the nuclear dye Hoechst 33342 (NR/Hoechst) after incubation of HepG2 cells in a medium with a high content of oleic (1 mM) and palmitic (0.5 mM) acids and the addition of 30 or 60 μM samples of pinostrobin and its solid dispersion within 24 hours

HepG2 cells	NR/Hoechst	
	I series of experiments	II series of experiments
Control	0.095±0.012	0.191±0.023
High fatty acid (FA) medium supplemented with pinostrobin sample solvent (dimethyl sulfoxide)	0.578±0.068*	0.755±0.053*
FA + Pinostrobin 30 μM	0.504±0.046	0.708±0.056
FA + Pinostrobin 60 μM	0.443±0.052#	0.562±0.029#
A medium with a high content of fatty acids (FA`) and the addition of a solvent sample of a solid dispersion of pinostrobin (water)	0.480±0.038*	0.785±0.044*
FA + pinostrobin solid dispersion 30 μM	0.453±0.064	0.725±0.073
FA + pinostrobin solid dispersion 60 μM	0.440±0.030	0.703±0.061

Notes: in all cases n = 6; FA – medium with a high content of oleic (1 mM) and palmitic (0.5 mM) acids and the addition of pinostrobin sample solvent (dimethyl sulfoxide); FA` – medium with a high content of oleic (1 mM) and palmitic (0.5 mM) acids and the addition of a solvent sample of a solid dispersion of pinostrobin (water); * - differences compared to "Control" wells are statistically significant; # - differences compared to "FA" wells are statistically significant; & - the differences compared to the "FA`" wells are statistically significant.

The studied sample of pinostrobin (1) had a slightly smaller effect on the intracellular accumulation of lipids induced by an environment with a high content of fatty acids. A statistically significant decrease in lipid levels in cells was recorded at a concentration of 60 μM and amounted to 23.4% (p<0.05) and 25.6% (p<0.05) in the first and second series of experiments, respectively (Table 2, Figure 8).

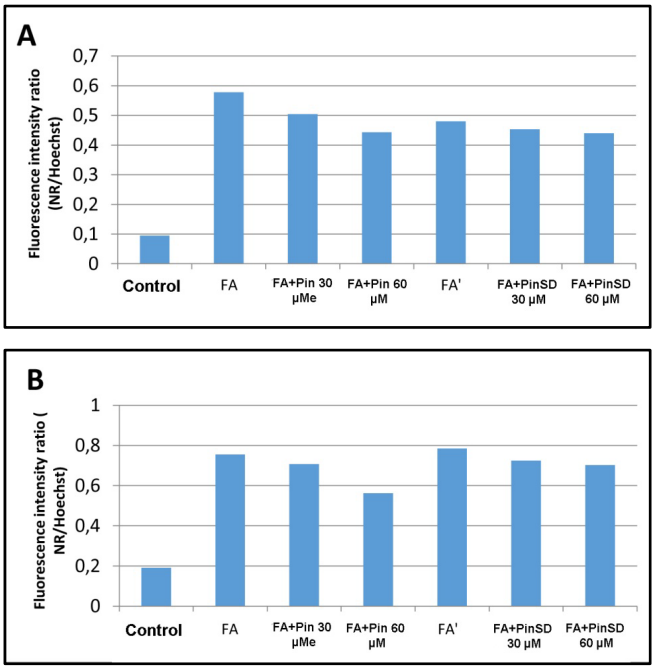


*Figure 8: Effect of pinostrobin samples and its solid dispersion on intracellular lipid accumulation in HepG2 cells induced by medium with increased content of oleic (1 mM) and palmitic (0.5 mM) acids during incubation for 24 h: A – first series of experiments; B – second series of experiments. Notes: in all cases n = 6; FA – medium with increased content of oleic (1 mM) and palmitic (0.5 mM) acids and addition of pinostrobin sample solvent (dimethyl sulfoxide); FA` – medium with increased content of oleic (1 mM) and palmitic (0.5 mM) acids and addition of pinostrobin solid dispersion sample solvent (water); * – differences compared to "Control" wells are statistically significant; # – differences compared to "FA" wells are statistically significant; & - differences compared to the "FA`" wells are statistically significant*

It is known that long-chain free fatty acids (mainly saturated) cause oxidative stress and increase the production of reactive oxygen species in cells, including the HepG2 lineage [28], [29].

It was found that lipotoxicity induced by incubation of HepG2 cells in a medium with a high content of fatty acids for 24 hours leads to a significant increase in the fluorescence of the probe for reactive oxygen species DCFDA by 3.1 times (p <0.001) and 2.9 times (p <0.001) in the first and second series of experiments, respectively (Table 3, Figure 9).

Table 3: HepG2 cells in a medium with a high content of oleic (1 mM) and palmitic (0.5 mM) acids and the addition of 30 or 60 μM pinostrobin samples and its solid dispersion within 24 hours

HepG2 cells	Intracellular fluorescence intensity of DCFDA	
	I series of experiments	II series of experiments
Control	446.2±34.8	444.8±19.3
Medium with a high content of fatty acids (FA) and the addition of a solvent for Leu and PN samples (dimethyl sulfoxide)	1375.6±82.8*	1301.9±65.0*
FA + pinostrobin 30 μM	1248.0±91.5	1227.6±70.8
FA + pinostrobin 60 μM	1092.0±86.5#	1091.9±64.4#
A medium with a high content of fatty acids (FA`) and the addition of a sample solvent, a solid dispersion of pinostrobin (water)	1265.3±146.0*	1355.2±48.2*
FA + pinostrobin solid dispersion 30 μM	1202.6±102.1	1281.3±40.8
FA + pinostrobin solid dispersion 60 μM	1199.0±95.1	1287.9±58.4

Notes: in all cases n = 6; FA – medium with a high content of oleic (1 mM) and palmitic (0.5 mM) acids and the addition of pinostrobin sample solvent (dimethyl sulfoxide); FA` – medium with a high content of oleic (1 mM) and palmitic (0.5 mM) acids and the addition of a solvent sample of a solid dispersion of pinostrobin (water); * - differences compared to "Control" wells are statistically significant; # - differences compared to "FA" wells are statistically significant; & - the differences compared to the "FA`" wells are statistically significant.

Pinostrobin (1) reduced the elevated level of intracellular reactive oxygen species only at the maximum concentration (60 μM), which was reflected in a decrease in DCFDA fluorescence in cells by 20.6% (p<0.05) and 16.1% (p<0.05) in the first and the second series of experiments, respectively. The water-soluble pinostrobin sample had no effect on free fatty acid-induced production of intracellular reactive oxygen species at all concentrations (Table 3, Figure 9).

At the same time, the solid dispersion of pinostrobin had no effect on the production of intracellular reactive oxygen species induced by free fatty acids at all concentrations.

The decrease in fluorescence of the probe for reactive oxygen species DCFDA in HepG2 cells during incubation in a medium with a high content of oleic (1 mM) and palmitic (0.5 mM) acids is associated with the antioxidant activity of the tested molecules and depends on their ability to overcome cell membranes and penetrate into the intracellular space.

Thus, the solid dispersion of pinostrobin in experiments in vitro has comparable toxicity to the parent pinostrobin molecule (1) and has a less pronounced hepatoprotective effect, which may be due to the limited number of pathways for transport of hydrophilic molecules of this class into the intracellular space. In contrast, the lipophilic parent molecule pinostrobin (1) enters cells by diffusion through the lipid bilayer and reduces levels of intracellular lipids and

reactive oxygen species in a concentration-dependent manner.

Thus, the solid dispersion of pinostrobin in in vitro experiments has comparable toxicity to the original pinostrobin molecule and a less pronounced hepatoprotective effect. This may be due to the limited number of ways for transporting the hydrophilic molecule of the solid dispersion of pinostrobin into the intracellular space. In contrast, the lipophilic parent molecule pinostrobin (1) enters cells by diffusion through the lipid bilayer and reduces levels of intracellular lipids and reactive oxygen species in a concentration-dependent manner.

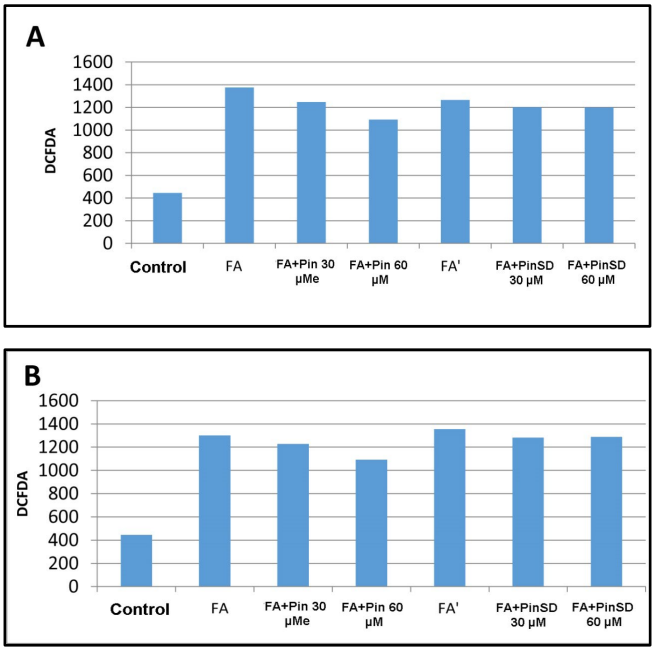


*Figure 9: Effect of pinostrobin samples and its solid dispersion on the production of reactive oxygen species in HepG2 cells induced by a medium with an increased content of oleic (1 mM) and palmitic (0.5 mM) acids during incubation for 24 h: A – the first series of experiments; B – the second series of experiments. Note: in all cases n = 6; FA – a medium with an increased content of oleic (1 mM) and palmitic (0.5 mM) acids and the addition of pinostrobin sample solvent (dimethyl sulfoxide); FA` – a medium with an increased content of oleic (1 mM) and palmitic (0.5 mM) acids and the addition of pinostrobin sample solvent and its solid dispersion (water); * - differences compared to the "Control" wells are statistically significant; # - differences compared to the "FA" wells are statistically significant; & - differences compared to the "FA`" wells are statistically significant*

At the same time, the results obtained in this in vitro experiment do not exclude a more pronounced hepatoprotective effect of water-soluble substances in vivo due to an increase in their bioavailability, which requires subsequent studies using laboratory animals.

A water-soluble solid dispersion of pinostrobin with the disodium salt of glycyrrhizic acid can be used to prevent and treat chronic inflammatory processes of the hepatobiliary system. The proposed mechanism of action of solid dispersion is similar to the original pinostrobin molecule (1), i.e., it interacts with lipid peroxides, captures free radicals and eliminates the inhibitory effect of lipoperoxides on the key enzyme of cholesterol catabolism in the liver - microsomal 7a-hydroxylase, as well as on lipoprotein lipase. All of the above is manifested by normalising the blood lipid profile.

Capillary strengthening activity

P-vitamin activity is associated with the action of flavonoids, which strengthens capillaries and reduce their permeability. The mechanism of action of substances with P-vitamin activity on the human body includes strengthening of capillaries, antioxidant effect, anti-inflammatory effect, and improvement of blood circulation. The P-vitamin activity of flavonoids is mainly associated with their effect on the enzymes hyaluronidase and cyclooxygenase, which play a key role in regulating vascular permeability and capillary strength.

Due to the relatively high antioxidant activity of pinostrobin (1) and its solid dispersion, it limits the processes of chain reactions of free radical oxidation, prevents excessive oxidation of lipids, proteins, and nucleic acids, and protects cell membranes from damage by oxidants. Consequently, pinostrobin (1) and its water-soluble solid dispersion can form the basis of effective drugs that allow you to maintain health and activity for many years.

That's why further research is directed at studying the influence of pinostrobin (1) and its solid dispersion on the permeability of rats' skin vessels. In the result of the research, it has been established that in animal control groups, most fast-painted papule caused zymosan for 54.8 sec, then painted papule in place introduction histamine (115.6 sec.), then - egg protein - 123.4 sec., and then papule painted formaldehyde - 228 sec. Introduction pinostrobin (1) and its solid dispersion slowed down colouring papules; hence, reduced permeability of blood vessels caused the introduction of chromogens. Thus, against the background applications, zymozana application pinostrobin (1) increased time staining papules by 1.38 times, and its solid dispersion - by 1.3 times, introducing diclofenac sodium increased time staining at 1.1 times, respectively.

Against the background of histamine use, the introduction of pinostrobin (0.5 ml/kg) increased the staining time of papules by 1.2 times, pinostrobin (1 ml/kg) - by 1.33 times, its solid dispersion (0.5 ml/kg) - by 1.22 times, its solid dispersion (1 ml/kg) - by 1.5 times, the introduction of diclofenac sodium increased the staining time by 1.1 times, respectively. Against the background of egg white use, the introduction of the test samples and the comparison drug increased the staining time of papules by 1.5, 1.6 times, 1.8 times, 1.6 times, 1.45 and 1.4 times, respectively. Against the background of formalin, the test compounds and the comparison drugs diclofenac sodium, pinostrobin and its solid dispersion, the staining time of papules

increased by 1.3, 1.36 times, 1.4 times, 1.5 times, 1.18 times and 1.13 times, respectively. In terms of the increase in papule staining time, the effectiveness of pinostrobin solid dispersion (1 ml/kg) is significantly higher than the effectiveness of pinostrobin (1) at the same dose.

Pinostrobin (1) and its solid dispersion have a capillary-strengthening and membrane-stabilizing effect, which is confirmed by a significant decrease in vascular permeability by 1.2-1.4 times. The membrane-stabilizing activity of pinostrobin (1) and its solid dispersion in the model of spontaneous hemolysis of erythrocytes was 31.3 - 40.7%. In this type of activity, they reliably exceeded the effect of the comparison drug diclofenac sodium. The maximum activity was observed when using pinostrobin solid dispersion at a dose of 1 ml/kg, which should be used as a conditionally therapeutic for further studies. It has been established that pinostrobin (1) and its solid dispersion prevent the destruction of cell membranes, strengthen the walls of blood vessels and capillaries, protect them from damage, and restore the permeability of the vessel walls and blood flow. Thus, their further use in the clinic for patients with arterial hypertension and retinopathy will allow them to obtain a pharmacological effect due to the normalization of the tone of the vascular wall and blood flow.

Anti-inflammatory activity

Several flavonoids have anti-inflammatory, analgesic, and immunoprotective properties in the presence of antioxidant activity. Due to their high complexing properties, they remove heavy metals from the body, including radio nuclides, help restore the tone of blood vessels, normalize the lipid spectrum of the blood, and slow down the development of atherosclerotic plaques [14, 30].

Based on literature data, we conducted our own studies of the anti-inflammatory activity of pinostrobin and its water-soluble solid dispersion.

Table 4 presents data about the development of oedema caused by TPA and experimental animals that received pinostrobin (1) and its solid dispersion with disodium salt of glycyrrhizic acid in doses 1.5, 2.0 and 3.0 mg in the ear. At 2 hours, there were no significant differences in ear thickness between groups. After 4, 6 and 24 hours, a statistically significant decrease ($p<0.001$) relative to TPA was observed in animals receiving pinostrobin and its solid dispersion at doses of 1.5, 2.0 and 3.0 mg per ear. Although inflammation increased over time in all groups, anti-inflammatory activity in the pinostrobin-treated groups remained statistically significant ($p < 0.05$). After 48 hours, there was a slight reduction in TPA-induced inflammation; however, the test substance, alone and its solid dispersion, continues to exhibit anti-inflammatory activity ($p < 0.001$).

Table 4: Anti-inflammatory activity of pinostrobin and its solid dispersion with respect to swelling caused TPA in experimental animals. Data is received through 2, 4, 6, and 24 hours

Samples	Dose, mg	2 hours	6 hours	24 hours	48 hours
Pinostrobin	1.5	0.20 ± 0.01	0.24 ± 0.01	0.25 ± 0.02	0.29 ± 0.02
Pinostrobin	2.0	0.20 ± 0.01*	0.21 ± 0.01*	0.26 ± 0.01*	0.29 ± 0.01*
Pinostrobin	3.0	0.19 ± 0.02**	0.20 ± 0.01**	0.25 ± 0.01**	0.27 ± 0.01**
Solid dispersion of pinostrobin with disodium salt of glycyrrhizic acid	1.5	0.18 ± 0.01***	0.21 ± 0.01***	0.24 ± 0.02***	0.25 ± 0.01***
Solid dispersion of pinostrobin with disodium salt of glycyrrhizic acid	2.0	0.17 ± 0.01**	0.21 ± 0.02**	0.24 ± 0.02**	0.29 ± 0.02**
Solid dispersion of pinostrobin with disodium salt of glycyrrhizic acid	3.0	0.18 ± 0.01***	0.20 ± 0.02***	0.21 ± 0.02***	0.25 ± 0.02***
Control	3.0	0.22 ± 0.01	0.27 ± 0.01	0.35 ± 0.02	0.39 ± 0.0

Table 4 shows that in animals that received pinostrobin only (1), the level of inflammation is comparatively higher than in animals receiving a solid dispersion of pinostrobin with disodium salt of glycyrrhizic acid through 4, 6 and 24 hours. In comparison indicators of oedema between two samples, it was noted that the solid dispersion of pinostrobin showed statistically significant anti-inflammatory activity for three used doses ($p < 0.001$).

Thus, the anti-inflammatory activity of the studied samples, associated with the impact on various links in the inflammatory reaction chain, is realised by inhibiting the induction of tissue inflammatory mediators - cytokines. As a result of the analysis of the dependence of the specific pharmacological activity of pinostrobin and its solid dispersion on their chemical structure and the presence of a complexing agent, individual structural fragments were identified, the presence of which determines both the antioxidant and anti-inflammatory potential of flavonoids.

Antiulcer activity

Visual studies showed a pronounced antiulcer activity of a solid dispersion of pinostrobin with the disodium salt of glycyrrhizic acid at the dose used; pinostrobin (1) also exhibited an antiulcer effect, but to a lesser extent.

A histological study of stomach tissue in a group of intact animals showed that the mucous wall of the cardinal section on the side of the oesophagus is lined with stratified squamous keratinising epithelium. In the area of transition to the cardinal section of the stomach, the villi of mucosal crypts, uniform in height, represent the transition to the cardinal section. In the submucosal layer of the gastric mucosa, there are glands of round and elongated shapes distributed evenly; single lymphocytes are detected in the lamina propria.

In the mucosa of the lesser curvature, the formation of an acute ulcer is noted, which is represented by a defect in its surface layers with desquamation and swelling of the mucosa. Characteristic signs of an acute alcoholic ulcer are the presence of a morphological picture of circulatory disorders at the level of the microvasculature in the form of vascular congestion, diapedetic and linear forms of haemorrhage, and severe vascular congestion. The vessels are thin walled with perivascular haemorrhages. Lymphoid infiltration was noted in the lamina propria of the mucous layer of the stomach wall. Predominantly lymphoid-leukocyte infiltration was detected in the perivascular zone in the submucosal layer (Figure 10 a, b, c, d).

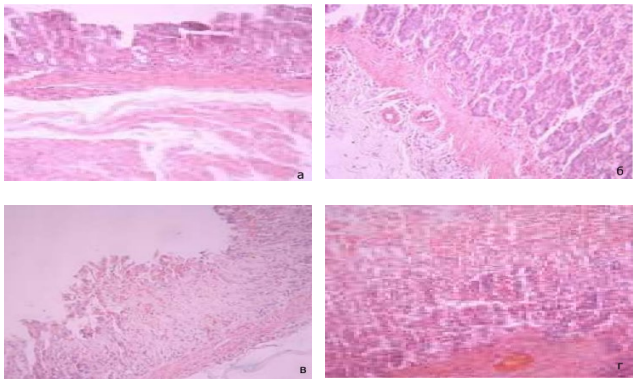


Figure 10: Acute alcoholic ulcer. Stomach, lesser curvature. Control: a – the formation of an acute ulcer with a defect and swelling of the mucous membrane, lymphoid infiltration of the lamina propria of the mucous layer of the stomach wall is noted; b – perivascular lymphoid-leukocyte infiltration of the submucosal layer; c – overview of an acute superficial ulcer with areas of defect and hemorrhage; d – (fragment) of hemorrhage in the area of an acute ulcer. Surround: hematoxylin and eosin. UV: a, b x200; c, d x100

In the case of acute alcoholic gastric ulcer in the cardinal part of the stomach in the control group of animals, necrobiotic changes in the mucous membrane, perivascular lymphoid-leukocyte infiltration with an admixture of eosinophils, swelling of the submucosal layer of the cardinal part of the stomach, and stratified squamous integumentary epithelium with keratinization were also noted (Figure 11 a, b).

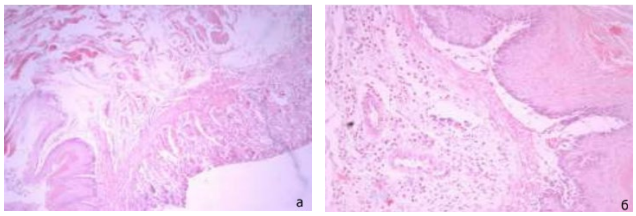


Figure 11: Acute alcoholic ulcer. Cardiac part of the stomach. Control: a – necrobiotic changes in the mucosa in acute gastric ulcers; b – perivascular lymphoid-leukocyte infiltration with an admixture of eosinophils and swelling of the submucosal layer of the cardia of the stomach. Staining: hematoxylin and eosin. Uv. a x200; b x400

When treated with pinostrobin solid dispersion in groups of experimental animals with reproduction of acute alcoholic ulcers, positive dynamics were noted. At the same time, the condition of the cardiac part of the gastric mucosa was characterised by the presence of moderate stromal oedema; no inflammatory reaction was noted (Figure 12 a, b).

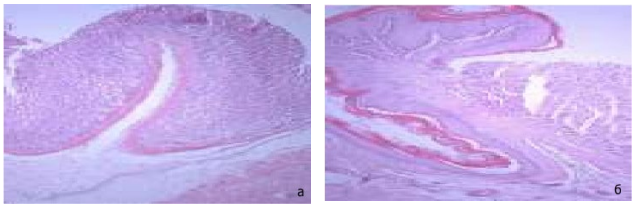


Figure 12: Acute alcoholic ulcer. Treatment with pinostrobin solid dispersion: a – mucous layer, moderate stromal edema, no inflammatory infiltration; b – cardiac part of the stomach, there is no inflammatory infiltration. Surround: hematoxylin and eosin. UV:a, b x200

However, in some rats, when treated with solid dispersion of pinostrobin, moderate infiltration with lymphocytes and stromal oedema were detected in the mucous layer; in the submucosal layer, moderate perivascular infiltration with lymphocytes and vascular congestion were detected (Figure 13 a, b).

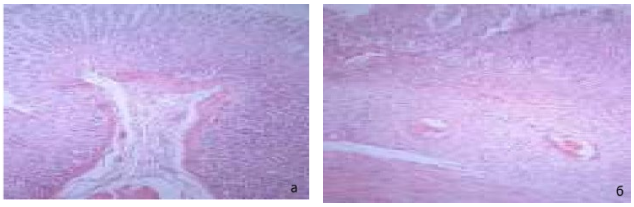


Figure 13: Acute alcoholic ulcer. Treatment with pinostrobin solid dispersion: a – in the mucous layer there is moderate infiltration of lymphocytes, stromal edema; b – in the submucosal layer there is moderate perivascular infiltration with lymphocytes, vascular congestion. Surround: hematoxylin and eosin. UV: a, b x200

When comparing the oedema scores between the two samples, it was noted that the pinostrobin solid dispersion showed anti-inflammatory activity higher than that of pinostrobin for the three doses used ($p < 0.001$).

The effectiveness of the solid dispersion of pinostrobin with the disodium salt of glycyrrhizic acid is characterised by a decrease not only in large, pinpoint, stripe-like ulcers but also in a decrease in the number of ulcerative lesions in laboratory animals in the experimental group. It is obvious that under the influence of the studied substances, hyperactivation of stress-releasing systems of the animal body is limited: hypothalamic-pituitary-adrenal, sympathoadrenal with a decrease in aggression factors, along with the mobilisation of protective factors of the gastric mucosa and stabilisation of cell membranes due to the action of a solid dispersion of pinostrobin with disodium salt of glycyrrhizic acid and pinostrobin (1).

Pinostrobin (1) and its solid dispersion have been shown to have significant protective effects against ethanol-induced gastric mucosal damage by scavenging ethanol-generated free radicals by activating cellular antioxidant defenses. Compound (1)

and its water-soluble solid dispersion can protect the gastric mucosa by reducing ulcer area and mucosal contents and reducing or eliminating submucosal edema and leukocyte infiltration. Thus, the gastrointestinal tract is the main organ of pinostrobin (1) and solid dispersion in relieving gastrointestinal diseases. Thus, a solid dispersion of pinostrobin with glycyrrhizic acid disodium salt can effectively reduce peptic ulcers.

Conclusions

Analysis of modern literature data and our own results of studying the biological activity of pinostrobin (1) and its solid dispersion indicates that this polyphenolic compound is a promising medicinal substance for preventing and complex therapy of various diseases. It should be noted that it has no toxic influences.

Pinostrobin (1) and its solid dispersion with the disodium salt of glycyrrhizic acid are promising research objects as a pharmacologically active substance with antioxidant, hepatoprotective, anti-inflammatory, and antiulcer effects.

The polar fragment of the pinostrobin molecule (1) - the phenolic hydroxyl group (cycle A) - gives it hydrophilic properties, and the prenyl fragment (cycle B) – has lipophilic properties. Upon contact with the cells of macro- and microorganisms, the molecule is distributed so that the lipophilic part penetrates the lipid shell of the membranes, and the hydroxylated phenolic part remains in the aqueous phase. This determines their selective interaction with various acceptors and affects the permeability of membranes.

The experiments conducted indicate that the pinostrobin molecule (1) and its solid dispersion, containing aromatic nuclei and an OH group in their structure, have antioxidant properties.

Our experiments with pinostrobin (1) and its solid dispersions of different structures allow us to conclude that compounds with an OH group at the C-5 position and a carbonyl group at the C-4 position increase the antioxidant activity in several of them.

It is possible that the mechanism of the antioxidant and hepatoprotective action of pinostrobin (1) and the solid dispersion based on it may be associated with the occurrence of such parallel processes as the scavenging of free radicals and the chelation of metals (Fe2+) of variable valence by inhibiting peroxide processes occurring with the participation of active forms of oxygen.

The pinostrobin molecule (1) has two pharmacophore centres: the first is the hydroxyl group at C-5 in ring A, which captures the radical; a second is a 4-oxo group, which is presumably capable of initiating the delocalisation of ring B electrons.

Because pinostrobin (1) has certain features in terms of its influence on the body's antioxidant defence system, it seems relevant to create combined and total medicinal substances based on this flavonoid, the use of which may result in an enhanced pharmacological effect.

The results of the experiments indicate that the antioxidant activity of pinostrobin and its solid dispersion is associated with its ability to bind free radicals and thereby influence the processes of lipid peroxidation in cell membranes, have a significant hepatoprotective effect, affecting the synthesis of proteins in the liver and prevent the phenomena of cholestasis, anti-inflammatory the effect associated with the impact on various links in the inflammatory reaction chain is limited by the hyperactivation of stress-releasing systems of the body, along with the mobilisation of protective factors of the gastric mucosa and the stabilisation of cell membranes.

Thus, after additional studies confirm the unique properties of pinostrobin, we can recommend its water-soluble form - a solid dispersion of pinostrobin with disodium glycyrrhizic acid as a prophylactic agent that prevents chronic fatigue syndrome and slows down aging, as well as a therapeutic agent in the complex treatment of diseases of various organs and systems.

Institutional Review Board Statement

All experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC). There is a conclusion of the Ethical Committee of the NJSC "Medical University of Karaganda" No. 9, Protocol No. 4 dated 12/06/2021.

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