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Hypolipidemic Activity of Solid Dispersion of Leucomisin

Sergazy M. Adekenov[1*], Dmitry Leontyevich Savchenko[1], Leyla Ibatullaevna Arystan[2]

[1]JSC "Research and Production Center "Phytochemistry", Karaganda, Kazakhstan; [2]NCJSC Karaganda Medical University, Karaganda, Kazakhstan

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***Correspondence:** Sergazy M. Adekenov. JSC "Research and Production Center "Phytochemistry", Karaganda, Kazakhstan. E-mail: arglabin@phyto.kz
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Abstract

BACKGROUND: The sesquiterpene lactone leucomisin is a promising compound with hypolipidemic activity, but it is practically insoluble in water, which reduces its bioavailability. Therefore, we synthesized a solid dispersion of leucomisin with the glycyrrhizic acid disodium salt, samples of which were studied for hypolipidemic activity.

AIM: To study the hypolipidemic activity of solid dispersion of leucomisin with glycyrrhizic acid disodium salt.

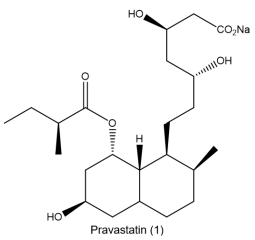
METHODS: We synthesized the solid dispersion of leucomisin by "simple mixing" method. The study of hypolipidemic activity of the samples was carried out according to known methods on models of acute tween hyperlipidemia, acute ethanol hyperlipidemia, fatty liver dystrophy of rats.

RESULTS: Based on the results of the experiments conducted, it was determined that the solid dispersion of leucomisin: Reduces triacylglycerols and cholesterol levels in rat serum in acute experimental hyperlipidemia induced by Tween-80, free fatty acids, serum triacylglycerols and triacylglycerol levels in rat liver in an ethanol-induced acute hyperlipidemia model; Lowers the level of triacylglycerols and increases the ratio of phospholipids to triacylglycerols in experimental fatty liver degeneration induced by tetrachloromethane in rats; Activates the antiperoxidation enzymes glutathione reductase and glutathione peroxidase and increases the redox potential of the glutathione system; and Reduces the activity of 3-hydroxy-3-methylglutaryl-coenzyme A reductase in rat liver and increases cholesterol excretion through the gastrointestinal tract.

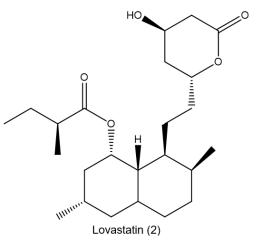
CONCLUSION: The synthesized solid dispersion of leucomisin showed pronounced hypolipidemic activity.

Introduction

In recent years, the prevalence of atherosclerotic cardiovascular disease has increased, which is attributed to the aging of the population and an increase in metabolic risk factors, including hyperlipidemia. Currently, pravastatin (1), lovastatin (2), fibrates and nicotinic acid are mainly used to treat hyperlipidemia. However, the above hypolipidemic agents are expensive and their long-term use causes side effects [1], [2], [3], [4], [5].



Pravastatin (1)



Lovastatin (2)

In this regard, natural compounds have attracted the attention of researchers due to their safety and therapeutic potential, as well as the ability to influence various targets during the development of diseases. One of the promising compounds with hypolipidemic activity is the sesquiterpene lactone leucomisin (3). Leucomisin is a major component of the extract of *Artemisia leucodes* Schrenk., which is widespread in Southern Kazakhstan, the exploitable stock of dry above-ground mass on the area of 11 hectares is 28 tonnes with the volume of annual procurement of 11.6 tonnes. Besides *Artemisia leucodes* Schrenk., sources of leucomisin are *Artemisia cana* ssp. viscidula Beetle, *Artemisia santolina* L., *Artemisia tridentata* ssp. tridentata, *Achillea millefolium* L [6], [7], [8], [9], [10], [11].

However, leucomisin (3) is practically insoluble in water, which significantly reduces the bioavailability of the drug substance in the body and, consequently,

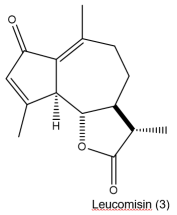
its pharmacological effect. One of the ways to solve this problem is solid-phase synthesis, which allows a significant increase in both water solubility and release of the active ingredient from the dosage form [12].

To solve the problem of water solubility of the studied sesquiterpene lactone, we synthesised a solid dispersion based on leucomisin with the glycyrrhizic acid disodium salt, samples of which were tested for hypolipidemic activity.

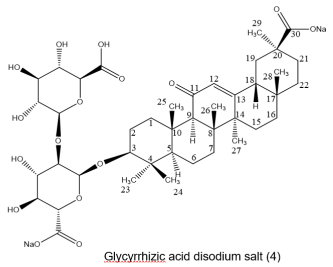
Materials and methods

Objects of study

The objects of study are solid dispersions of leucomisin (3) with glycyrrhizic acid disodium salt (4) with a substrate to carrier ratio of 1:5, obtained by the “simple mixing” method [13].



Leucomisin (3)



Glycyrrhizic acid disodium salt (4)

Leucomisin (3), a colorless crystalline substance of composition C15H18O3, mp. 196-198°C, [α] D 20 +56 °, is a major component of CO2 extract of *Artemisia leucodes* Schrenk (yield 1.63%, based on dry raw materials).

Glycyrrhizic acid disodium salt (4), gray mustard-colored powder with composition C40H62O16Na2, mp. 152-156°C, found in licorice roots used in the synthesis of solid dispersion as a carrier.

Methods for studying lipid-lowering activity

A model of acute Tween hyperlipidemia was induced by intraperitoneal administration of the detergent Tween-80 at 2 g/kg [14]. The levels of triacylglycerols and total cholesterol were estimated in blood by the fermented method using kits from Cronolab AG (Switzerland).

The model of acute ethanol hyperlipidemia was induced by ethyl alcohol at a dose of 5 g/kg of absolute ethanol [14]. The level of free fatty acids (FFA), triacylglycerol (TAG) and total cholesterol in the blood was determined by an enzymatic method using kits from Cronolab AG (Switzerland) and Randox (UK). Lipids were extracted from the liver according to the method of J. Folch [15], in which the level of primary products of lipid peroxidation (LPO) - diene conjugates [16] and the content of triacylglycerols (TAG) were estimated by the fermented method [17]. In liver homogenates, the level of total (GSH + GSSG), reduced (GSH) and oxidized (GSSG) glutathione was determined by the cyclic method [18] and the activity of antiperoxide defense enzymes – glutathione peroxidase (GPx) [19] and glutathione reductase (GR) [20].

The model of fatty liver degeneration of rats induced by tetrachloromethane (TCM) at a dose of 1 ml/kg of 1:1 (v/v) solution in vegetable oil on the 1st, 4th and 7th days of the experiment. In blood, the activity of marker enzymes - alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was determined by enzymatic method using kits from Cronolab AG (Switzerland), as well as the level of TBK-active products [21]. From the liver according to the method of J. Folch [15], lipids were extracted, in which the level of diene conjugates [16], lipid hydroperoxides by FOX 2 method [22] and the content of TAG and FL by enzymatic method [17] were evaluated. In liver homogenates, the levels of GSH+GSSG, GSSG and GSH were determined by the method of M.E. Anderson [18] and the activity of antiperoxide defense enzymes GPx [19] and GR [20].

Study of the antiradical activity of leucomisin solid dispersion in vitro was carried out using known methods: interaction with the stable radical diphenylpetrylhydrazyl (DPPH), interaction with the superoxide radical anion O2 •- , interaction with the hydroxyl radical OH- [23].

Results and discussion

Study of the lipid-lowering activity of leucomisin solid dispersion on a model of chronic alcoholism

Lipid metabolism disorders in chronic alcohol consumption have been of great interest in recent years. There are many works devoted to the study of the content of certain classes of lipoproteins in chronic alcohol intoxication [24], [25].

According to experimental data of M.I. Selevich when rats systematically consume alcohol for 3-4 weeks, they show an increase in the content of total lipids in the liver and blood serum [26].

In order to determine the hypolipidemic activity, we studied the effect of solid dispersion of sesquiterpene lactone leucomisin on lipid metabolism in rats with ethanol dyslipidemia.

Table 1: Average weight of organs after autopsy after 8 months of alcoholism (g)

Organs	Control	Rats are "alcoholics"
Heart	1.1 ± 0.04	1.46 ± 0.3
Lungs	2.6 ± 0.4	2.4 ± 0.5
Liver	11.5 ± 0.3	13.6 ± 0.7
Spleen	1.4 ± 0.1	1.4 ± 0.2
Kidneys	2.2 ± 0.01	2.48 ± 0.2
Stomach	1.67 ± 0.06	2 ± 0.1
Brain	2.02 ± 0.07	2 ± 0.01
Testes	3.3 ± 0.1	3.4 ± 0.3

Body weight indicators were initially: in the experimental group 172.8 ± 21.6 g, in the control group 174.2 ± 20.2 g. After 8 months. in the experimental group – 192.2 ± 38.6; in the control group – 246 ± 22.2 g. Thus, the weight gain in the experimental group was 11.2%, in the control group – 41.2%, i.e. there was a lag in body weight gain in “alcoholic” rats.

Indicators of the average weight of rat organs after autopsy after 8 months of alcoholization are shown in Table 1, and data from studying the lipid spectrum before and after alcoholization are shown in Table 2.

Table 2: Lipid spectrum indicators after 8 months of alcoholization

No./p	Groups of animals	OL, g/l	cholesterol, mg/dl	TG, mg/dl	HDL, mol/l
1	Control (n = 6)	8.9 ± 0.9	59.9 ± 7.5	50.5 ± 15.6	0.47 ± 0.05
2	Alcoholic rats (n = 36)	9.53 ± 1.5	66.2 ± 23.1	123.3 ±13.6*	0.07 ± 0.01**
Note: * - significance of differences with control at p<0.01; ** - at p<0.001					

As can be seen from Table 2, after 8 months of alcoholization in the experimental group there was a significant increase in the content of triglycerides and a sharp decrease in the level of high-density lipoproteins in the blood serum. Cholesterol levels also trended higher. This suggests that long-term ethanol intoxication in rats leads to a persistent disturbance of lipid metabolism, which is in good agreement with the literature data on the toxic effect of ethanol on the functional ability of the liver, which is the main regulator of lipid metabolism in the body [18], [19], [20], [21].

After 8 months of regular consumption of ethanol, the rats of the experimental group were divided into 2 subgroups: one of them was intragastrically administered a solid dispersion of leucomisin at a dose of 10 mg/kg in 1% starch mucus for 6 weeks, the other was transferred to a normal drinking regimen.

The results of the effect of oral administration of solid dispersion of leucomisin are shown in Table 3.

As shown in Table 3, administration of leucomisin solid dispersion for 6 weeks resulted in a significant reliable decrease in total lipids, triglycerides and an increase in the level of antiatherogenic high-density lipoproteins (HDL). Cholesterol levels were practically unchanged in both groups.

Table 3: Lipid metabolism indicators after 6 weeks of administration of solid leucomisin dispersion

№	Groups of animals	OL, g/l	cholesterol, mg/dl	TG, mg/dl	HDL, mol/l
1	Alcoholic rats without treatment (n=12)	8.23 ± 1.2	68.6 ± 4.3	120 ± 12.2	0.06 ± 0.02
2	Alcoholic rats + leucomisin solid dispersion 10 mg/kg (n=24)	2.28 ± 0.24*	65.2 ± 7.3	92.05 ± 7.42*	0.25 ± 0.03**
Note: * - significance of differences between groups at p<0.01; ** - at p<0.001					

Based on the above data, it can be concluded that prolonged alcoholization leads to a persistent disorder of lipid metabolism, expressed as an increase in the content of total lipids, triglycerides and atherogenic lipoproteins in the blood serum of experimental animals.

The application of leucomisin solid dispersion for 6 weeks has a positive effect on lipid metabolism, restoring the normal ratio of lipid fractions.

The main effect of acute and chronic alcohol consumption, which affects many types of metabolism, is a disturbance in the redox state of the liver (NADH:NAD ratio). The conversion of ethanol to acetaldehyde (the major metabolite) and the latter to acetate or acetyl-coenzyme A is accompanied by an increase in the NADH/NAD ratio within hepatocytes. Thus, ethanol metabolism contributes to the shift of redox processes in liver cells towards reductive reactions, which leads to suppression of protein synthesis, increases lipid peroxidation and reduces carbohydrate, lipid and other forms of intermediate metabolism [27].

The involvement of NAD in ethanol metabolism makes its effects on other types of metabolism understandable due to the competitive inhibition that occurs. Alcohol and acetaldehyde are involved in oxidative processes in the body, competing for enzymes and cofactors. The main role in the enhancement of lipid peroxidation belongs to the excessive formation of oxygen-containing free radicals during ethanol oxidation, and as a result of direct induction of NADH-oxidase O2-generating system and microsomal NADP-dependent lipid peroxidation, which is one of the mechanisms of structural and functional rearrangement of biological membranes [28], [29].

Oxidative stress is a key element in the pathogenesis of liver damage from ethanol. The introduction of ethanol causes lipid peroxidation either by increasing the production of reactive oxygen species or by reducing the content of endogenous antioxidants. The role of ethanol-induced cytochrome P450, located in microsomes, and cytosolic xanthine oxykidase enzymes in these processes, has been experimentally established [30].

The morphological stages of alcoholic liver damage have been well studied both experimentally and clinically. The initial link, along with ultrastructural changes, is protein and fatty degeneration of the liver. The main role in the development of fatty hepatosis belongs to lipid metabolism disorders both in hepatocytes and in the body as a whole. Already a single dose of ethanol is accompanied by an increase in blood lipid levels. This is due, firstly, to the mobilization of fat from fat depots due to the stimulating effect of alcohol on the sympathoadrenal system and, secondly, to the acceleration of the synthesis of fatty acids in the liver [27].

The consequence of endoplasmic reticulum hyperplasia is increased synthesis of cholesterol and lipoproteins. In turn, the hydrolysis of cholesterol esters and the catabolism of cholesterol into bile acids, on the contrary, are slowed down.

The results of a morphological study of the internal organs of rats in the control group that did not receive ethanol showed that there were no deviations in the structure of the organs.

On sections of the liver, hepatic lobules with a vein located in the center were clearly visible; hepatic beams, consisting of hepatocytes with a well-defined cellular structure, were located radially around the vein.

After 8 months of alcoholization in rats, hemorrhages and severe liver degeneration were noted. In addition, when exposed to alcohol, cholesterol deposits in the aorta develop in the form of lipid stains, while no such changes were observed in the control group.

When treated with leucomisin solid dispersion for 6 weeks, the intensity of alcoholic hepatitis and the number of cholesterol spots in the intima of the aorta gradually decreased.

Study of the lipid-lowering activity of leucomisin solid dispersion on a model of alloxan diabetes

The experiment was carried out on 35 non-linear male rats weighing 276 ± 5.2 g. The animals were kept on a standard vivarium diet, with free access to water and food in accordance with the rules adopted by the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific goals. Diabetes was induced by intraperitoneal administration of alloxan monohydrate (Sigma, USA) at a dose of 90 mg/kg for 4 days. The development of diabetes was monitored by glucose levels taken from the tail vein. Animals with glucose levels more than 15 mmol/l were selected into experimental groups.

The glucose content in rats before the experiment was 7.8 ± 0.2 mmol/l. 7 days after the last administration of alloxan, a persistent increase in glucose in the blood of rats was observed in 15 animals and amounted to 28.9 ± 3.1 mmol/l, 10 rats died, 5 did not develop diabetes (glucose level 8.2 ± 1.2 mmol /l).

After the development of diabetes, the rats were divided into 2 groups. The 1st control group (n = 7) was left without treatment, the rats of the second group (n = 8) were intragastrically administered a solid dispersion of leucomisin at a dose of 10 mg/kg for 4 weeks. Group 3 – intact rats. Table 4 shows the dynamics of glucose and lipids in 3 experimental groups before and 4 weeks after the administration of leucomisin. As can be seen from the table, in the intact group the cholesterol level at the end of the experiment was 53.5 ± 10.1 mg/dl, and in rats with diabetes it was 120.2 ± 18.3 mg/dl; triglyceride level 37.4±12 and 19.1±8.5 mg/dL; high density lipoproteins (HDL) – 12.4±2.6 to 23.6±9 mg/dl, respectively; low-density lipoprotein (LDL) level – 33.6±9.4 and 90.8 mg/dl, respectively. Thus, in rats with a model of alloxan diabetes, hypercholesterolemia and dyslipidemia reliably develop with an increase in the level of lipoprotein NP and the atherogenicity index (AI).

Table 4: Indicators of carbohydrate and lipid metabolism in rats with experimental diabetes mellitus after 4 weeks of administration of solid leucomisin dispersion

Groups	Glucose, mmol/l	Cholesterol, mg/dl	Triglycerides, mg/dl	HDL, mg/dl	LDL, mg/dl	AI
1	2	3	4	5	6	7
Intact (n = 5)	7.8 ± 0.2	53.5 ± 10.1	37.4 ± 12	12.4 ± 2.6	33.6 ± 9.4	3.4 ± 0.7
Control (diabetes mellitus without treatment) (n = 7)	29.4 ± 2.9 *	120.2 ± 18.3 *	19.1 ± 8.5	23.6 ± 9.1	90.8 ± 16.8 *	5.1 ± 1.5 *
Experience (diabetes mellitus + leucomisin solid dispersion) (n = 8)	28.5 ± 3.4*	95.3 ± 9.6+	16.1 ± 5.5	23.3 ± 3.2	70.2 ± 7.5+	3.2 ± 0.6+

Note: * – significance of differences with intact rats; + – significance of differences with the control group.

As shown in Table 4, in the experimental group, as a result of a 4-week intake of solid leucomisin dispersion, the cholesterol level significantly decreased compared to the control group and amounted to 95.3 ± 9.6 mg/dl, although it did not reach the level in the intact group (53, 5 ±10.1).

A similar trend was observed in LDL levels. Thus, after induction of diabetes, there was a significant increase in LDL levels to 90.8±16.8 mg/dL. After taking leucomisin solid dispersion, this indicator decreased significantly and amounted to 70.2±7.5 mg/dl.

The most significant indicator reflecting lipid metabolism disorders is the atherogenic index (AI). As can be seen from Table 4, AI in intact rats was 3.4±0.7 mg/dL. Against the background of developing diabetes, it increased and amounted to 5.1±1.5. And after correction with solid leucomisin dispersion, it decreased to the level of intact rats.

Study of the hypolipidemic effect of leucomisin solid dispersion on models of acute hyperlipidemia

A model of acute Tween hyperlipidemia was induced by administration of the detergent Tween-80 intra-abdominal at 2 g/kg [14]. The animals were divided into 3 groups: Group I – control group; in Group II, Tween 80 2 g/kg i.a. was administered; in Group III, against the background of Tween, animals were administered intragastrically a solid dispersion of leucomisin at a dose of 10 mg/kg.

The level of triacylglycerols and total cholesterol in the blood was assessed by an enzymatic method using kits from Cronolab AG (Switzerland).

After the administration of Tween-80, in Group II there was a statistically significant increase in lipids: triacylglycerol by 153%, total cholesterol by 22% compared to the control group (Table 5).

Table 5: Effect of a course administration of solid leucomisin dispersion (10 days, 10 mg/kg per day) on the content of triacylglycerol (TAG) and total cholesterol in the blood serum of rats with acute hyperlipidemia induced by Tween-80 2 g/kg (M ± m, n = 6)

Indicators	I Control	II Twin-80 2 g/kg i.a.	III Solid dispersion of leucomisin + Tween 80 2 g/kg i.p.
Content TAG, mM	0.989 ± 0.124	2.500 ± 0.204 153%, P<0.01 (II-I)	1.194 ± 0.104 +20%, P>0.05 (III-I) -52%, P<0.01 (III-II)
Total cholesterol content, mM	1.69 ± 0.06	2.07 ± 0.04 +22%, P<0.05 (II-I)	1.74 ± 0.07 +3%, P>0.05 (III-I) -16%, P<0.05 (III-II)

At the same time, a course of administration of a solid dispersion of leucomisin for 10 days at a dose of 10 mg/kg (Group III) significantly prevented the increase in lipids: TAG by 52%, total cholesterol by 16% compared to the group that did not receive the drug.

In evaluating the effect of leucomisin solid dispersion on triacylglycerol (TAG) and free fatty acid (FFA) levels, a model of hyperlipidemia induced by ethanol administration was reproduced. The results are summarized in Table 6.

As can be seen from Table 6, ethanol administration resulted in a dramatic increase in serum FFA and TAG (118% and 251%, respectively) in rats of Group II compared to the control. At the same time, there was also an increase in the level of triacylglycerol (TAG) and diene conjugates (DC) in liver tissue.

Table 6: Effect of a course administration of solid dispersion of leucomisin (10 days, 10 mg/kg) and nicotinic acid (10 days, 25 mg/kg) on the level of triacylglycerol (TAG) and free fatty acids (FFA) in blood serum, as well as the content triacylglycerol and diene conjugates in the liver of rats with experimental ethanol-induced hyperlipidemia (M ± m, n = 6)

Indicators	I Control	II Ethanol 5 g / kg	III Solid dispersion of leucomisin 10 mg /kg + ethanol 5 g /kg	IV Nicotinic acid 25 mg /kg + ethanol 5 g /kg
1	2	3	4	5
Concentration of FFA in blood serum, mM	0.563 ± 0.046	1.230 ± 0.104 +118% p<0.01 (II - I)	0.605 ± 0.080 +7%, p>0.05 (III-I) -51% p<0.01 (III-II)	0.502 ± 0.047 -eleven%, p>0.05 (IV-I) -59% p<0.01 (IV-II)
TAG content in blood serum, mM	0.66 ± 0.07	2.32 ± 0.23 +251% p<0.01 (II - I)	1.37 ± 0.09 +108%, p<0.01 (III-I) -40% p<0.01 (III-II)	1.14 ± 0.11 +72%, p<0.05 (IV-I) -51% p<0.01 (IV-II)
TAG level in the liver, mg/g liver	4.35 ± 0.53	11.66 ± 1.56 +168% p< 0.01 (II - I)	6.70 ± 0.33 +54% p<0.05 (III-I) -43% p<0.01 (III-II)	6.20 ± 0.43 +43%, p<0.05 (IV-I) -47% p<0.01 (IV-II)
Diene conjugates, OD_{223} /g liver	1.13 ± 0.13	1.76 ± 0.08 +56% p< 0.01 (II - I)	1.77 ± 0.10 +57% p>0.05 (III-I) +1% p<0.01 (III-II)	1.32 ± 0.09 +17% p<0.05 (IV-I) -43% p<0.01 (IV-II)

Administration of the well-known hypolipidemic drug nicotinic acid (Group IV) led to a decrease in serum FFA concentration to the level of the control group and statistically significantly reduced TAG levels.

Administration of leucomisin led to a decrease in the level of FFA in the serum by 50%, and the content of TAG by 40% compared to Group II. There was an almost 2-fold decrease in the level of TAG in the liver.

The next step was to study the effect of a course administration of a solid dispersion of leucomisin and nicotinic acid on the content of total, reduced, oxidized glutathione and the activity of glutathione peroxidase and glutathione reductase in the liver of rats with experimental hyperlipidemia induced by ethanol.

Table 7: Effect of a course administration of solid dispersion of leucomisin (10 days, 10 mg/kg) and nicotinic acid (10 days, 25 mg/kg) on the content of total, reduced, oxidized glutathione and the activity of glutathione peroxidase and glutathione reductase in the liver of rats with experimental hyperlipidemia, induced by ethanol, (M± m, n = 6)

Indicators	I Control	II Ethanol 5 g / kg	III Solid dispersion of leucomisin 10 mg /kg + ethanol 5 g /kg	IV Nicotinic acid 25 mg /kg + ethanol 5 g /kg
1	2	3	4	5
GSH + GSSG , µmol /g liver	6.92 ± 0.80	2.46 ± 0.28 -64%, p < 0.01 (II-I)	4.62 ± 0.15 -33%, p<0.01 (III-I) +87%, p<0.01 (III-II)	3.54 ± 0.14 -49%, p<0.01 (IV-I) +44%, p<0.05 (IV-II)
GSH, µmol /g liver	6.45 ± 0.79	2.19 ± 0.27 -66%, p < 0.01 (II-I)	4.48 ± 0.16 -31% p<0.01 (III-I) +105%, p<0.01 (III-II)	3.33 ± 0.14 -48%, p<0.01 (IV-I) +52%, p<0.01 (IV-II)
GSSG µmol /g liver	0.47 ± 0.04	0.27 ± 0.01 -43%, p < 0.01 (II-I)	0.14 ± 0.02 -70%, p<0.01 (III-I) -48%, p<0.01 (III-II)	0.22 ± 0.02 -53%, p<0.01 (IV-I) -19%, p=0.11 (IV-II)
GSH/GSSG	13 , 1	8.2	36.6	15.7
GPx activity , µmol NADPH $_2$ / min * mg protein	0.458 ± 0.017	0.362 ± 0.007 -20%, p < 0.05 (II-I)	0.467 ± 0.040 +2%, p >0.05 (III-I) +27%, p<0.01 (III-II)	0.443 ± 0.011 -3%, p >0.05 (IV-I) +21%, P<0.01 (IV-II)
GR activity , nmol GSSG / min * mg protein	53.4 ± 1.2	33.6 ± 3.3 - thirty%, p<0.01 (II-I)	58.6 ± 4.1 +8%, p>0.05 (III-I) +54%, p<0.01 (III-II)	44.4 ± 1.9 -17%, P < 0.05 (IV-I) +18%, p=0.05 (IV-II)

As follows from Table 7, in ethanol hyperlipidemia there is a significant decrease in total, reduced and oxidized glutathione, as well as a decrease in the activity of antioxidant defense enzymes - glutathione peroxidase (GPO), and glutathione reductase (GR). At course administration of solid dispersion of leucomisin the content of total glutathione increased by 87%, reduced by 105%, oxidized by 48% in comparison with the ethanol hyperlipidemia group that did not receive the drug. Activity of GPO and GR on the background of leucomisin solid dispersion administration increases by 27% and 54% and by their absolute values do not differ from the indicators of the control group.

Thus, we have established that the solid dispersion of leucomisin has an antioxidant effect in vivo, which may be due to the activation of antiperoxide defense enzymes.

Evaluation of the effect of leucomisin solid dispersion on spontaneous and adrenaline-stimulated lipolysis in serum and isolated rat adipocytes showed that administration of leucomisin solid dispersion has a dose-dependent inhibitory effect on lipolysis in rat serum, which is expressed as a decrease in free fatty acids (FFA) both before and after adrenaline stimulation, and the effect is comparable to that of nicotinic acid administration.

In the incubation medium of adipocytes, a reduced content of free fatty acids is also observed when a solid dispersion of leucomisin is administered at a dose of 50 mg/ml, comparable to the effect of nicotinic acid.

Study of the hypolipidemic effect of solid leucomisin dispersion on a model of fatty hepatosis induced by carbon tetrachloride

Tables 8-9 present the results of studying the hypolipidemic and antioxidant effects of solid dispersion of leucomisin on the model of carbon tetrachloride fatty degeneration. As can be seen from the tables, when carbon tetrachloride hepatosis is induced, the content of triglycerides increases 5 times, and the content of phospholipids in liver tissue decreases 3 times. The activity of the cytolysis enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) is also significantly activated. Against the background of course application of solid dispersion of leucomisin the activity of cytolysis enzymes is markedly reduced (17-20% lower) in comparison with the group without treatment.

Table 8: Effect of a course administration of solid leucomisin dispersion (10 mg/kg, 8 days) on the content of triacylglycerol (TAG), phospholipids (PL) and the PL/TAG ratio in the liver of rats with carbon tetrachloride (TCM)-induced fatty degeneration

Indicators	I Control	II Leucomisin solid dispersion 10 mg /kg	III CCl_4 1 ml/ kg solution in vegetable oil 1:1 (v / v)	IV Solid dispersion of leucomisin 10 mg /kg + CCl_4 1 ml/ kg solution in vegetable oil 1:1 (v / v)
TAG content, mg/g liver	4.14 ± 0.42	2.48 ± 0.20 -40 % P<0.01 (II-I)	25.86 ± 0.68 + 523%, P < 0.01 (III - I)	15.76 ± 1.43 +281%, P<0.01 (IV-I) -39%, P<0.05 (IV-III)
Phospholipid content, mg/g liver	21.01 ± 2.47	18.72 ± 1.08 -eleven%, P =0.09 (II-I)	14.99 ± 1.34 -29%, P < 0.01 (III - I)	14.814 ± 0.48 -29%, P<0.01 (IV-I) -1%, P>0.05 (IV-III)

The content of lipid peroxidation (LPO) products in blood serum with the introduction of carbon tetrachloride increases as follows: TBA-active products - by 75%; diene conjugates by 144%, lipid hydroperoxides by 66%.

With a course of administration of a solid dispersion of leucomisin, a statistically significant decrease in peroxidation products occurs in the group of rats with fatty hepatosis compared to the group that did not receive the drug.

Table 9: Effect of a course administration of solid dispersion of leucomisin (10 mg/kg, 8 days) on the activity of liver transaminases and the content of lipid peroxidation products (LPO) in the blood serum of the liver of rats with carbon tetrachloride (TCM)-induced fatty degeneration

Indicators	I Control	II Leucomisin solid dispersion 10 mg /kg	III CCl_4 1 ml/ kg solution in vegetable oil 1:1 (v / v)	IV Solid dispersion of leucomisin 10 mg /kg + CCl_4 1 ml/ kg solution in vegetable oil 1:1 (v / v)
ALT, U/l	44.5 ± 2.3	19.7 ± 1.7 -56 % P<0.01 (II-I)	1023.7 ± 52.3 +2200%, P<0.01 (III-I)	847.0 ± 33.9 +1803%, +1803%, P<0.01 (IV-I) -13%, P<0.05 (IV-III)
AST, U/l	52.9 ± 5.4	47.7 ± 1.9 -10% , P>0.05 (II-I)	113.7 ± 53 +115%, P<0.01 (III-I)	90.8 ± 5.3 +91%, P<0.01 (IV-I) -20%, P<0.01 (IV-III)
Content of TBA-active products in blood serum, μM / l	3.30 ± 0.15	2.76 ± 0.11 - 16 % , P<0.05 (II-I)	5.76 ± 0.20 +75%, P<0.01 (III-I)	3.26 ± 0.25 -1%, P>0.05 (IV-I) -13%, P<0.05 (I.V-III)
Diene conjugates, nmol / mg PL	2.55 ± 0.15	1.53 ± 0.32 -40%, P <0.05 (II-I)	6.22 ± 0.37 +144%, P <0.01 (III-I)	4.62 ±0.28 +81%, P<0.01 (IV-I) -26%, P=0.05 (IV-III)
Lipid hydroperoxides, nmol / g liver	5584 ± 409	4492 ± 224 - 20 % , P<0.05 (II-I)	9258 ± 329 +66%, P<0.01 (III-I)	6124 ± 440 +10%, P>0.05 (IV-I) -34%, P<0.01 (IV-III)

A study of the effect of a course administration of a solid dispersion of leucomisin (10 mg/kg, 8 days) on the content of total, reduced, oxidized glutathione and the activity of glutathione peroxidase and glutathioproductase in the liver of rats with carbon tetrachloride-induced fatty degeneration showed the following: against the background of an 8-day administration of a solid dispersion leucomisin restores the reduced level of total glutathione to control values. The activity of TPO and GR enzymes statistically significantly increases compared to the group without treatment, approaching the level of control figures (Table 10).

Table 10: Effect of a course administration of solid leucomisin dispersion (10 mg/kg, 8 days) on the content of total, reduced, oxidized glutathione and the activity of glutathione peroxidase and glutathione reductase in the liver of rats with carbon tetrachloride (TCM)-induced fatty degeneration

Indicators	I Control	II Leucomisin solid dispersion 10 mg /kg	III CCl_4 1 ml/ kg solution in vegetable oil 1:1 (v / v)	IV Solid dispersion of leucomisin 10 mg /kg + CCl_4 1 ml/ kg solution in vegetable oil 1:1 (v / v)
1	2	3	4	5
GSH + GSSG , μmol /g liver	7.56 ± 0.61	8.06 ± 0.71 +7% P>0.05 (II - I)	5.43 ± 0.52 -28% P < 0.0 1 (III - I)	8.19 ± 0.50 +8%, P>0.05 (IV-I) +51%, P=0.01 (IV-III)
GSH, μmol /g liver	6.85 ± 0.65	7.77 ± 0.79 +13% P>0.05 (II - I)	4.79 ± 0.57 - thirty % P < 0.0 5 (III - I)	7.58 ± 0.57 +11%, P>0.05 (IV-I) +58%, P<0.01 (IV-III)
GSSG μmol /g liver	0.72 ± 0.13	0.28 ± 0.03 +61% P<0.01 (II - I)	0.64 ± 0.05 - eleven % P > 0.0 5 (III - I)	0.61 ± 0.05 -15%, P>0.05 (IV-I) -5%, P>0.05 (IV-III)
GSH/GSSG	11 , 7	285	7 , 7	13.4
GPx activity , μmol $NADPH_2$/ min*mg protein	0.467 ± 0.05	0.540 ± 0.033 + 16% P>0.05 (II - I)	0.334 ± 0.012 -28% P < 0.0 5 (III - I)	0.423 ± 0.020 -9%, P>0.05 (IV-I) +27%, P<0.01 (IV-III)
GR activity , nmol GSSG / min*mg protein	52.9 ± 5.7	56.3 ± 5.5 +6 % P>0.05 (II - I)	31.7 ± 2.7 - 40 % P < 0.0 1 (III - I)	43.1± 2.9 -19%, P>0.05 (IV-I) +36%, P<0.05 (IV-III)

Thus, the solid dispersion of leucomisin has an antioxidant effect in vivo, due to the activation of antiperoxide protection enzymes and an increase in the

redox potential of the glutathione system in the liver of rats, which may contribute to the hypolipidemic effect of the sesquiterpenoid.

The effect of a course of use of a solid dispersion of leucomisin and rosuvastatin calcium on the level of cholesterol in the blood serum and liver of rats

According to experimental data, administration of a solid dispersion of leucomisin to rats at a dose of 10 mg/kg for 10 days inhibits the activity of the key enzyme of cholesterol biosynthesis - HMG-CoA reductase in the blood serum and liver of rats. The effect produced was comparable to the reference drug rosuvastatin calcium.

Since the main mechanism of action of statins is inhibition of the enzyme HMG-CoA reductase, we assessed the effect of a solid dispersion of leucomisin on the activity of this enzyme, which showed that with the introduction of the reference drug rosuvastatin calcium, the activity of HMG-CoA reductase decreased by 50%, and with the introduction of a solid dispersion leucomisin by 32%, which was a statistically significant difference with the indicators of the control group without administration of drugs.

Thus, administration of a solid dispersion of leucomisin to rats at a dose of 10 mg/kg for 10 days inhibits the activity of the key enzyme in cholesterol biosynthesis HMG-CoA reductase in rat liver. At the same time, under the influence of sesquiterpene lactone, cholesterol excretion increases.

Conclusions

Based on the results obtained, we formulated the following conclusions:

A course of administration of a solid dispersion of leucomisin at a dose of 10 mg/kg for 10 days reduces the level of triacylglycerols and cholesterol in the blood serum of rats with acute experimental hyperlipidemia induced by Tween-80.

Meanwhile, solid dispersion of leucomisin:

• Exerts hypolipidemic effects in ethanol-induced acute hyperlipidemia models by reducing the levels of free fatty acids, triacylglycerols in serum and triacylglycerol in rat liver.

• Reduces the level of free fatty acids in the blood serum by inhibiting spontaneous and stimulated lipolysis in adipose tissue, which may be one of the mechanisms of the lipid-lowering action of the sesquiterpenoid.

• Reduces the level of triacylglycerols and increases the ratio of phospholipids to triacylglycerols in experimental fatty liver degeneration induced by carbon tetrachloride in rats.

• Activates antiperoxide enzymes glutathione reductase and glutathione peroxidase and increases the redox potential of the glutathione system.

• Reduces the activity of HMG-CoA reductase in the liver of rats and increases the excretion of cholesterol through the gastrointestinal tract.

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