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PEROXIDE PROCESSES AND BIOSYNTHESIS OF CHOLESTEROL DERIVATIVES IN RABBIT TISSUES AT ACUTE L-ARGININE-INDUCED PANCREATITIS AND ITS CORRECTION

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Abstract

The positive corrective effect of fed flaxseed oil on the condition of the pancreas in acute L-arginine-induced pancreatitis was shown, the development of which was assessed by the number of necrotized acinar epitheliocytes in the head and tail of the pancreas and the activity of lipase and α-amylase in blood plasma. Feeding sunflower oil does not show a similar corrective effect. The normalizing effect of fed flaxseed oil on the state of the antioxidant defense system in rabbits in acute L-arginine-induced pancreatitis, on the content of thiobarbituric acid-positive products and the activity of superoxide dismutase, catalase and glutathione peroxidases in blood. Feeding sunflower oil leads to a deterioration of the oxidative-prooxidant balance. The ability of fed flaxseed oil to prevent disorders of content of non-esterified and esterified cholesterol in blood plasma, liver and skeletal muscle of rabbits in acute L-arginine-induced pancreatitis has been established. Feeding sunflower oil under the above conditions impairs the lipid composition of rabbit tissues. The positive effect of fed flaxseed oil on the ratio of anti-inflammatory polyunsaturated fatty acids of the ω-3 family to proinflammatory polyunsaturated fatty acids of the ω-6 family in the fatty acid spectrum of esterified cholesterol in blood plasma, liver and skeletal muscle of rabbits in acute L-arginine-induced pancreatitis. The result of the fed sunflower oil action on the studied indicators is quite the opposite. In acute L-arginine-induced pancreatitis, feeding flaxseed oil stimulates the conversion of cholesterol to bile acids, 25-OH-vitamin D_3, testosterone, aldosterone and cortisol in rabbits. The stimulating ability of fed sunflower oil in this regard is less pronounced.

Key words*: acute arginine pancreatitis, correction, derivatives of cholesterol, fatty acids, lipids, rabbits.*

INTRODUCTION

Factors protecting the pancreas of humans and animals from their own digestion include: 1) synthesis of proteolytic and lipolytic enzymes in an inactive state, their isolation from the cytosol of cells in zymogenic granules during maturation (Eydoux, 2006); 2) stable connection of enzymes and inhibitors, which keeps them inactive; 3) the specificity of the action of active lipases only in relation to triacylglycerols in the emulsified state, which are not present in acinar cells (Human & Jain, 2001); 4) protection of acinar cells from the reflux of pancreatic juice, the possibility of its exit into the interstitial space and lymphatic capillaries (Chen et al., 1999); 5) the presence in the blood nonspecific factors of proteolytic enzymes inactivation – α_2-macroglobulin and α_1-antitrypsin (Morgado et al., 2005). The effects of pro-inflammatory cytokines such as IL-1, IL-6, IL-8, TNF-α in humans and animals are inhibited by such "anticytokines" as IL-1 receptor antagonists or soluble receptors (soluble TNF-receptor-55, soluble TNF-receptor-75) (Bozza et al., 2011; Tverdokhlib et al., 2011). Together with some anti-inflammatory cytokines (IL-4, IL-10), these endogenous anticytokines form the basis for a fragile balance between pro-inflammatory and anti-inflammatory mediators (Ansell & Hawthorne, 2000; Puppo et al., 2001). It should be noted that proinflammatory eicosanoids (certain groups of prostaglandins) in humans and animals are synthesized from

polyunsaturated fatty acids of the family ω-6 (linoleic, eicosatrienic, eicosatetraenoic-arachidonic, docosatetraenoic) (Rivis et al., 1997; Jorristma et al., 2001). Therefore, the above mentioned polyunsaturated fatty acids are called pro-inflammatory. Polyunsaturated fatty acids of the family ω-3 (linolenic, eicosapentaenoic, docosatrienic, docosapentaenoic, docosahexaenoic) in humans and animals are precursors of anti-inflammatory eicosanoids (Ang et al., 2006; Ang et al., 2009). In addition, polyunsaturated fatty acids of the family ω-6 (linoleic, eicosatrienic, eicosatetraenoic-arachidonic, docosatetraenoic) stimulate the synthesis of proinflammatory cytokines, and polyunsaturated fatty acids of the family ω-3 (linolenic, eicosapentaenoic, docosacotrienic, docosapentaenoic, docosahexaenoic) stimulate the synthesis of anti-inflammatory ones (Curley, 1996). The pathogenesis of acute pancreatitis in humans and animals is based on damage to the pancreas by its own enzymes and the development of systemic inflammatory response syndrome (Joshi et al., 2005; Wang & Chan, 2015). Acute pancreatitis in humans and animals develops on the background of gallstone disease, chronic alcohol poisoning (Makhija and Kingsnorth, 2002), traumatic and burn injuries (Chypre et al., 2012), surgery in the bio-pancreato-duodenal area (Schmidt et al., 1992), the use of various drugs and poisons (Zanotti et al., 2015), infectious and parasitic diseases (Rasilainen et al., 2002), tumor obstructions and atherosclerotic lesions of the vascular system (Rollins et al., 2006). In order to study the pathogenetic and metabolic aspects of acute pancreatitis, various methods of its experimental reproduction using small laboratory animals have been proposed. These include direct administration of calcium chloride solution to pancreatic tissue (Drogomyretska et al., 2010), intraperitoneal administration of L-arginine solution (Posokhova & Bukovska, 2002; D**e Roos** et al., 2009), injection of ethyl alcohol into the common bile duct (Drogomyretska, 2010), temporary ligation of the bile and pancreatic ducts (Jayaraman et al., 2011) and so on. Currently, there are two versions of the mechanism of acute pancreatitis with intraperitoneal administration of L-arginine. One version indicates that due to its strong activation of oxide-nitrate synthase and the formation of nitrogen oxide, which, in excessive formation, with superoxide anion-radical produces peroxynitrite, which damages the lipids of cell membranes (Vlasov et al., 2011; Akon & Min, 2013). In another version, L-arginine promotes the overproduction by the pancreas of enzymes that digest the gland itself (Yin, 2013). Regardless of the etiology, acute pancreatitis in humans and animals manifests itself in two forms: edematous and destructive (Shchipunov, 1996). In order to study certain aspects of acute pancreatitis use small laboratory animals with edematous form (Pereyaslov et al., 2000). In laboratory animals with edematous form of acute pancreatitis, the activity of lipolytic blood enzymes increases (Sweiry & Mann, 1996). At the same time in their blood the level of reactive forms of Oxygen, primary and secondary products of lipid peroxidation, sharply increases (Datsenko et al., 2000; Guicciardi, 2005). The above is observed on the background of a decrease in the concentration of fat-soluble vitamins, which are involved in the non-enzymatic chain of antioxidant protection (Donaldson, 1979). At the same time in the blood of laboratory animals with edematous form of acute pancreatitis decreases the content of trace elements (Zinc, Manganese, Selenium), which activate the enzymatic link of antioxidant protection, primarily sureroxide dismutase and glutathione peroxidase (Donaldson, 1979; Neoptolemus & Bhuani, 2006). On this background, the activity of lipase and the content of non-esterified cholesterol in their blood increases (Makhija & Kingsnorth, 2002). Changes in the activity of individual enzymes and the content of lipids in the blood of laboratory animals with edematous form of acute pancreatitis are accompanied by changes in the concentration of lipids and fatty acids in the tissues of the whole organism (Pereyaslov, 2001). However, in the absence of sound research methods, changes in the content of lipids and fatty acids in the tissues of laboratory animals with edematous form of the acute pancreatitis are insufficiently studied. They are fragmentary in nature.

The purpose of the research was to investigate the effect of flaxseed oil to prevent pathological changes in the pancreas, disorders of oxidative-prooxidant balance and composition of lipids

and fatty acids and stimulate the conversion of cholesterol into appropriate derivatives in the tissues of rabbits with acute L-arginine-induced pancreatitis.

MATERIALS AND METHODS

The experiments were conducted in the vivarium of Danylo Halytsky Lviv National Medical University on male rabbits of the Gray Giant breed weighing 3.8-4.0 kg. Animals were divided into four groups (5 rabbits each): I - control (K); II - animals with experimental L-arginine-induced acute pancreatitis (P); III - animals with experimental L-arginine-induced acute pancreatitis, which was fed flaxseed oil (P+flaxseed oil); IV - animals with experimental L-arginine-induced acute pancreatitis, which was fed sunflower oil (P+sunflower oil).

Rabbits of all groups received standard granulated feed in the amount of 225 g/head/day for one month and without restrictions drinking water. However, during this period, rabbits of group P+flaxseed oil received daily compound feed with flaxseed oil (manufacturer "Elit-Pharm", Dnipro city, Ukraine), while rabbits of group P+sunflower oil received daily compound feed with sunflower oil (manufacturer "MACHNO", Dnipro city, Ukraine) at the rate of 1 ml/kg of body weight. In addition, 5 days before the end of the experiment, rabbits of group K were administered once intraperitoneally 2 ml/kg of body weight of saline solution Sodium chloride, and rabbits of groups P, P+flaxseed oil and P+sunflower oil (in the same amount of saline solution) - L-arginine at a dose of 4 g/kg of body weight. At the end of the experiment, the experimental rabbits after sampling blood from the ear vein under ether anesthesia were killed by decapitation. Blood, pancreas, liver and skeletal muscle samples were used as test material.

All animal interventions and slaughter were carried out in compliance with the requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes (Strasbourg, 1985) and the decision of the First National Congress on Bioethics (Kyiv, 2001).

Histological examinations of the pancreas were performed following Tverdokhlib et al. (2011). The number of necrotized acinar epitheliocytes in the head and tail of the pancreas was assessed. Lipase activity (KE 3.1.1.3) and α-amylase (KE 3.2.1.1) were determined in blood plasma. Lipase activity was determined by the chemical method described by Vlizlo et al. (2012) and α-amylase activity – using a standard set of reagents ("α-Amylase", "Filisit-Diagnostic", Ukraine).

According to the methods described by Vlizlo et al. (2012), the content of lipid peroxidation products, namely diene conjugates, lipid hydroperoxides and thiobarbituric acid-positive products, was determined in the blood plasma, liver and skeletal muscles of rabbits. In addition, in erythrocytes, liver and skeletal muscle of rabbits we determined the activity of the main enzymes of antioxidant protection – superoxide dismutase (KE 1.15.1.1), catalase (KE 1.11.1.6) and glutathione peroxidase (KE 1.11.1.9). The protein concentration in the test material was determined by Lowry.

The concentration of non-esterified and esterified cholesterol was determined in the blood plasma, liver and skeletal muscles according to the methods of Rivis et al. (2017). The fatty acid composition of the latter was determined by extraction of total lipids, their chromatography in a thin layer of silica gel, development of plates in Iodine vapor, isolation of the esterified cholesterol fraction and preparation from it fatty acid methyl esters by transesterification. It was determined total content of saturated fatty acids with pair (caprylic - 8:0, capric - 10:0, lauric - 12:0, myristic - 14:0, palmitic - 16:0, stearic - 18:0, arachinoic - 20:0) and the odd (pentadecanic - 15:0) number of Carbon atoms in the chain. It was determined also monounsaturated fatty acids of the families ω-7 (palmitoleic - 16:1) and ω-9 (oleic - 18:1 and eicosaenoic-20:1) and polyunsaturated fatty acids of the families ω-3 (linolenic - 18:3, eicosapentaenoic - 20:5, docosatrienic - 22:3, docosapentaenoic - 22:5 and docosahexaenoic - 22:6) and ω-6 (linoleic - 18:2, eicosatrienic - 20:3, eicosatetraenoic-arachidonic - 20:4, docosadienic - 22:2 and docosatetraenoic - 22:4). The ratio of polyunsaturated fatty acids of the ω-3 family to polyunsaturated fatty acids of the ω-6 family was determined also.

Obtained by the above method, methyl esters of fatty acids were introduced into the evaporator

of the gas-liquid chromatographic apparatus[41]. For studies of methyl esters of fatty acids we used gas-liquid chromatographic apparatus "Chrom-5" (Laboratorni pristroye, Praha) with stainless steel column 3700 mm in length and internal diameter of 3 mm. The column was filled with Chromaton-N-AW, 60-80 mesh, silanized HMDS (hexamethyldisilizan), coated with polydiethylene glycol adipinate (fixed liquid phase) in the amount of 10%. The consumption of carrier gas, chemically pure and dried Nitrogen (mobile phase) through the column at an inlet pressure of 1.5 x 10^5 Pa was about 65 ml/min. Burning flame was provided with hydrogen (25 ml/min) and air (380 ml/min). The isothermal mode of operation of the filled column with a polar liquid phase was kept at the 196^0C, evaporator and detector - 245^0C. Detector was of flame-ionizing type as the most sensitive. Recording of analysis results – differential. The column efficiency determined by McNair and Bonelli for the conventional mean peak on the chromatogram, palmitic acid methyl ester, was 1917 ± 110 theoretical plates. Peak identification on the chromatogram was performed by the method of "Carbon numbers" calculation as well as using chemically pure, standard, hexane solutions of fatty acid methyl esters.

The concentration of bile acids in the serum of animals was determined by fluorometric method after their separation by chromatography on paper according to Gromashevskaya et al. (1971). The content of vitamins (25-OH-vitamin D_3), androgens (testosterone) and corticosteroles (aldosterone and cortisol) in blood plasma was determined by enzyme-linked immunosorbent (solid phase) method (Vlizlo et al., 2012). Moreover, the content of 25-OH-vitamin D_3 was determined using the test system "Immunodiagnostic", and hormones content - with "DRG" reagents (Germany).

Obtained digital material was processed by the method of variation statistics using Student's criterion (Lopach et al., 2001). The arithmetic mean values (M), the arithmetic mean error (± m) and the probability of differences between the investigated arithmetic mean values (P) were calculated. Changes were considered probable at $P<0.05$. A special computer program Microsoft Exel for Windows XP was used for the calculations.

RESULTS AND DISCUSSIONS

An increase in the number of necrotized acinar epitheliocytes in the head and tail of the pancreas of rabbits with acute L-arginine-induced pancreatitis was found (Table 1). These data indicate the development of an inflammatory process in the pancreas and significant damage to its cells. This may be due to the fact that L-arginine is the main substrate of the enzyme NO-synthase and therefore increases the synthesis of Nitrogen oxide (Ang et al., 2009). The latter, in case of excessive formation, together with the superoxide anion radical, produces peroxynitrite (Posokhova & Bukovska, 2002), which in free radical oxidation reactions is able to oxidize and damage the lipid bilayer of cell membranes (Yaremchuk & Posokhova, 2011).

Feeding flaxseed oil, which contains in its composition according to our data 65.1% of anti-inflammatory linolenic acid, is able to correct the condition of the pancreas in rabbits with acute L-arginine-induced pancreatitis. In particular, the number of necrotized acinar epitheliocytes in the head and tail of the pancreas of rabbits is normalized in acute L-arginine-induced pancreatitis corrected by feeding flaxseed oil. The number of necrotized acinar epitheliocytes in the head and tail of the pancreas of rabbits increases sharply in acute L-arginine-induced pancreatitis and feeding sunflower oil, which also contains, according to our data, 61.8% of proinflammatory linoleic acid.

The activity of lipase and α-amylase in the blood plasma of rabbits increases significantly in acute L-arginine-induced pancreatitis. This is a consequence of inflammatory processes in the acinar cells of the pancreas, which activate exocrine cells that secrete a large number of hydrolytic enzymes into the blood. The latter with excessive activity are able to "digest" the tissues of the pancreas.

The activity of lipase and α-amylase in the blood plasma of rabbits in acute L-arginine-induced pancreatitis, corrected by fed flaxseed oil, is normalized. Fed sunflower oil, on the contrary, intensifies the lipase and α-amylase activity of rabbit blood plasma in acute L-arginine-induced pancreatitis.

It was found that in the blood plasma, liver and skeletal muscles of rabbits with acute L-arginine-induced pancreatitis, compared with the control, the concentration of primary and secondary products of lipid peroxidation – diene conjugate, lipid hydroperoxides and thiobarbituric acid-positive products increases significantly (Table 2). This is due to the fact that inflammatory processes in the pancreas cause oxidative stress of a systemic nature (Chuklin et al., 2011).

Feeding flaxseed oil normalizes and sunflower oil increases the concentration of primary and secondary products of lipid peroxidation in blood plasma, liver and skeletal muscle of rabbits in acute L-arginine-induced pancreatitis. The activity of antioxidant enzymes such as superoxide dismutase and glutathione peroxidase in rabbit red blood cells, liver and skeletal muscle in acute L-arginine-induced pancreatitis is greatly increased due to changes in the content of free radicals. In this biological material, catalase activity is significantly reduced. The results of our research are consistent with the literature (Biradar & Veeresh, 2013).

Feeding flaxseed oil normalizes and sunflower oil increases the concentration of primary and secondary products of lipid peroxidation in blood plasma, liver and skeletal muscle of rabbits in acute L-arginine-induced pancreatitis. The activity of antioxidant enzymes such as superoxide dismutase and glutathione peroxidase in rabbit red blood cells, liver and skeletal muscle in acute L-arginine-induced pancreatitis is greatly increased due to change in the content of free radicals. In this biological material catalase activity is significantly reduced. The results of our research are consistent with the literature (Biradar & Veeresh, 2013).

Fed flaxseed oil normalizes, and sunflower oil - increases the activity of superoxide dismutase, glutathione peroxidase and reduces catalase activity in erythrocytes, liver and skeletal muscle of rabbits in acute L-arginine-induced pancreatitis. Changes in superoxide dismutase, catalase, and glutathione peroxidase activity in rabbit red blood cells in acute L-arginine-induced pancreatitis and feeding of flaxseed and sunflower oils may be related to changes in the functional state of erythrocyte membranes. Apparently, the change in their activity was also influenced by the increased release of myeloid cells from the red bone marrow, which led to a change in the population composition of erythrocytes by age.

Acute L-arginine-induced pancreatitis showed an increase in esterified cholesterol in the blood plasma, liver and skeletal muscles of rabbits (Table 3). In addition, the concentration of non-esterified cholesterol increases in the blood plasma and liver of rabbits under conditions of pathology, which may be due to the inhibition of its conversion into the corresponding derivatives - bile acids, 25-OH-vitamin D_3, testosterone and corticosterols (Tsyupko, 2008).

Feeding flaxseed oil to rabbits with acute L-arginine-induced pancreatitis normalizes non-esterified and esterified cholesterol levels in the blood plasma, liver and skeletal muscle of rabbits. This reduces the level of non-esterified cholesterol in the liver and skeletal muscles of rabbits, which may be associated with its more intensive conversion into the corresponding derivatives (Neoptolemus & Bhuani, 2006).

Sunflower oil, fed to sick animals, aggravates the pathological condition and unbalances the content of non-esterified and esterified cholesterol in blood plasma, liver and skeletal muscle. In particular, the increase of non-esterified and esterified cholesterol in the blood plasma, liver and skeletal muscle of rabbits in acute L-arginine-induced pancreatitis and feeding sunflower oil may be associated with a decrease of their esterification and conversion into appropriate derivatives.

The above data may indicate a positive effect of feeding flaxseed oil, because the use of its fatty acids normalizes the processes of lipid metabolism (Neoptolemus & Bhuani, 2006; De Roos et al., 2009; Drogomyretska et al., 2010).

It was found that in the fatty acid composition of esterified cholesterol of blood plasma, liver and skeletal muscle of rabbits with acute L-arginine-induced pancreatitis increases the relative content of saturated fatty acids with even and odd number of Carbon atoms in the chain and monounsaturated fatty acids of ω-7 and ω-9 families, but decreases - polyunsaturated fatty acids of the families ω-6 and, especially, ω-3 (Tables 4, 5 and 6). However, in esterified cholesterol of blood plasma, liver and skeletal muscle of rabbits in acute L-arginine-induced

pancreatitis decreases the inclusion of more long-chained and more unsaturated derivatives of linoleic and linolenic acids.

In the fatty acid spectrum of blood plasma esterified cholesterol, liver and skeletal muscle of rabbits with acute L-arginine-induced pancreatitis corrected by fed flaxseed oil, the relative level of monounsaturated fatty acids of the ω-9 family decreases, but increases – polyunsaturated fatty acids of the ω-3 family (Tables 4, 5 and 6). The above leads to an increase in the ratio of polyunsaturated fatty acids of the family ω-3 to polyunsaturated fatty acids of the family ω-6. Along with that in the esterified cholesterol of blood plasma, liver and skeletal muscle of rabbits in acute L-arginine-induced pancreatitis, corrected with fed flaxseed oil, increases the inclusion of more long-chained and more unsaturated derivatives of linolenic and linoleic acids.

In the fatty acid composition of esterified cholesterol in blood plasma, liver and skeletal muscle of rabbits in acute L-arginine-induced pancreatitis and feeding sunflower oil, compared with the control, the relative content of saturated fatty acids with even amount of Carbon atoms in chain and polyunsaturated fatty acids of the ω-6 family, but decreases content of monounsaturated fatty acids of the ω-9 family and, especially, polyunsaturated fatty acids of the ω-3 family (Tables 4, 5 and 6). The above leads to a sharp decrease in the ratio of polyunsaturated fatty acids of the ω-3 family to polyunsaturated fatty acids of the ω-6 family.

Polyunsaturated fatty acids of the ω-3 family, compared with polyunsaturated fatty acids of the ω-6 family, have a more pronounced targeted effect on humans and animals through prostaglandins, thromboxanes and leukotrienes (Flaming & Kelly, 2004; Tsyupko, 2008). Polyunsaturated fatty acids of the ω-3 family in humans and animals stimulate the synthesis of such direct anti-inflammatory factors as cytokines IL-4, IL-10 (Trukhan, 2000). In addition, synthesized from them more long-chained and more unsaturated fatty acids are precursors of prostaglandins E_2, $F_{1\alpha}$, which are also anti-inflammatory factors, but already direct (Datsenko et al. 2000; De Roos et al., 2000; Wang & Chan, 2015).

At the same time, polyunsaturated fatty acids of the ω-6 family in humans and animals stimulate the synthesis of such direct proinflammatory factors as cytokines IL-1, IL-6, IL-8, TNF-α.

The ratio of anti-inflammatory polyunsaturated fatty acids of the ω-3 family to polyunsaturated fatty acids of the ω-6 family in the fatty acid spectrum of esterified cholesterol in blood plasma, liver and skeletal muscle of rabbits in acute L-arginine-induced pancreatitis is significantly reduced. In acute L-arginine-induced pancreatitis, corrected by feeding flaxseed oil, this ratio increases significantly, and with feeding sunflower oil to sick animals - it decreases significantly. That is, fed sunflower oil significantly exacerbates the disease of animals.

Esterified cholesterol, rich in polyunsaturated fatty acids, especially of the ω-3 family, in human and animal tissues is a precursor of bile acids, corticosteroles, 25-OH-vitamin D_3, estrogens and androgens (Smolyar, 2003). Polyunsaturated fatty acids of the ω-3 family, compared to polyunsaturated fatty acids of the ω-6 family, convert cholesterol into a less crystalline compound (Flaming & Kelly, 2004). Such cholesterol in metabolic processes penetrates better through cell membranes and is much less deposited in the walls of blood vessels. In general it can be stated that in relation to humans and animals, cholesterol esterified with polyunsaturated fatty acids of the family ω-3, has less atherogenic properties (Drogomyretska et al., 2010).

It has been recorded that in rabbits with high plasma, liver and skeletal muscle content of esterified cholesterol, rich in saturated and monounsaturated fatty acids, but poor in polyunsaturated, the concentration of taurocholic, glycocholic, glycodeoxycholic, cholic and deoxycholic acids in blood serum and 25-OH vitamin D_3 in blood plasma decreases (Table 7).

Table 1. The number of necrotized acinar epitheliocytes in the head and tail of the pancreas and lipase/α-amylase activity of rabbit blood plasma in acute L-arginine-induced pancreatitis + feeding flaxseed and sunflower oils (M ± m, n=5)

Material and indicators	Groups of rabbits			
	К	Р	Р+flaxseed oil	Р+sunflower oil
	The number of necrotized acinar epitheliocytes in the pancreas,%			
Head	5.2±0.2	24.1±1.1*	5.1±0.1	26.4±1.1*
Tail	1.6±0.1	14.5±1.3*	1.8±0.2	16.0±1.3*
	Activity of lipase (units/l) and α-amylase (Megaunits/l) in blood plasma			
Lipase	5.9±0.3	13.5±0.4*	6.0±0.4	15.7±0.5*
α-Amylase	73.8±1.6	120.5±2.9*	71.8±1.8	131.4±2.7*

Note: hereinafter * – the difference is probable in comparison with group K.

Table 2.The content of lipid peroxidation products and the activity of the main enzymes of antioxidant protection in the blood, liver and skeletal muscle of rabbits in acute L-arginine-induced pancreatitis and its correction (M ± m, n=5)

Researched indicators and units of measurement	Rabbits' groups			
	К	Р	Р+flax-seed oil	Р+sunflo-wer oil
	Blood plasma			
Diene conjugates. μmol/l	4.3±0.1	8.4±0.2*	4.5±0.1	9.3±0.2*
Lipid hydroperoxides. units E480/ml	1.3±0.1	4.9±0.2*	1.5±0.1	5.5±0.2*
Thiobarbituric acid-positive products. nmol/ml	3.5±0.1	5.8±0.2*	3.7±0.1	6.5±0.2*
	Liver			
Diene conjugates. μmol/l	88.2±1.6	135.0±4.2*	90.3±1.2	140.7±3.1*
Lipid hydroperoxides. units E480/ml	1.3±0.1	3.5±0.1*	1.4±0.1	4.1±0.1*
Thiobarbituric acid-positive products. nmol/ml	4.9±0.3	9.8±0.4*	5.2±0.3	10.6±0.5*
	Skeletal muscles			
Diene conjugates. μmol/l	86.4±1.5	131.3±2.0*	88.3±1.5	141.0±2.9*
Lipid hydroperoxides. units E480/ml	1.5±0.1	3.7±0.2*	1.6±0.1	4.2±0.2*
Thiobarbituric acid-positive products. nmol/ml	3.5±0.1	7.0±0.3*	3.8±0.1	7.8±0.3*
	Erythrocytes			
Superoxide dismutase. conventional units/mg protein	1.2±0.1	3.3±0.1*	1.2±0.1	3.4±0.1*
Glutathione peroxidase. mmol GSH/min mg protein	39.6±0.1	42.7±0.4*	39.7±0.1	43.4±0.2*
Catalase. mmol H_2O_2/min mg protein	4.3±0.1	3.7±0.1*	4.2±0.1	3.6±0.1*
	Liver			
Superoxide dismutase. conventional units/mg protein	22.3±0.3	29.6±0.3*	22.9±0.2	31.7±0.4*
Glutathione peroxidase. mmol GSH/min mg protein	3.3±0.1	4.6±0.1*	3.4±0.1	4.8±0.1***
Catalase. mmol H_2O_2/min mg protein	7.4±0.3	4.6±0.2*	7.0±0.3	4.2±0.2*
	Skeletal muscles			
Superoxide dismutase. conventional units/mg protein	19.5±0.4	23.9±0.4*	20.1±0.4	25.3±0.5*
Glutathione peroxidase. mmol GSH/min mg protein	5.8±0.1	8.6±0.1*	6.0±0.1	9.0±0.1*
Catalase. mmol H_2O_2/min mg protein	1.6±0.1	0.9±0.1*	1.5±0.1	0.8±0.1*

Table 3. The content of non-esterified and esterified cholesterol in blood plasma, liver and skeletal muscle of rabbits in acute L-arginine-induced pancreatitis by feeding flaxseed and sunflower oils (M ± m, n=5)

Cholesterol classes	Groups of rabbits			
	К	Р	Р+flaxseed oil	Р+sunflower oil
	Blood plasma. g/l			
Unesterified cholesterol	0.20±0.01	0.25±0.01*	0.18±0.01	0.27±0.01*
Esterified cholesterol	0.83±0.06	1.01±0.01*	0.79±0.06	1.05±0.01*
	Liver. g/kg of raw weight			
Unesterified cholesterol	2.73±0.03	3.06±0.09*	2.60±0.01*	3.20±0.06*
Esterified cholesterol	5.95±0.17	6.46±0.03*	5.64±0.15	7.30±0.26*
	Skeletal muscle. g/kg raw weight			
Unesterified cholesterol	0.88±0.03	0.94±0.03	0.74±0.01*	1.03±0.04*
Esterified cholesterol	2.79±0.05	3.04±0.04*	2.68±0.05	3.13±0.06*

Table 4. Fatty acid spectrum of esterified cholesterol in blood plasma of rabbits in acute L-arginine-induced pancreatitis + flaxseed/sunflower oils feeding, % (M ± m, n=5)

Fatty acids and their code	Rabbits' groups			
	К	Р	Р+flaxseed oil	Р+sunflower oil
Caprylic, 8:0	0.16±0.01	0.21±0.01*	0.17±0.01	0.23±0.01*
Caprynic, 10:0	0.22±0.01	0.28±0.01*	0.24±0.01	0.30±0.01*
Lauric, 12:0	0.30±0.01	0.31±0.01	0.32±0.01	0.35±0.01*
Myristic, 14:0	0.49±0.01	0.60±0.02*	0.52±0.01	0.62±0.02*
Pentadecanic, 15:0	0.30±0.01	0.36±0.01*	0.32±0.01	0.30±0.01
Palmitic, 16:0	7.45±0.12	8.28±0.16*	7.62±0.11	8.52±0.19*
Palmitoleic, 16:1	0.96±0.02	1.07±0.03*	1.00±0.03	1.08±0.06
Stearinic, 18:0	10.54±0.30	11.73±0.06*	9.99±0.39	13.44±1.08*
Oleic, 18:1	36.65±0.63	38.74±0.79*	34.26±0.85*	32.31±0.24*
Linoleic, 18:2	12.37±0.38	11.16±0.10*	12.03±0.40	13.21±0.15*
Linolenic, 18:3	5.44±0.10	4.94±0.06*	5.93±0.06*	4.80±0.12*
Arachinic, 20:0	0.35±0.01	0.44±0.01*	0.37±0.01	0.50±0.02*
Eicosaenoic, 20:1	0.21±0.01	0.23±0.01	0.19±0.01	0.24±0.01*
Eicosadienoic, 20:2	0.30±0.01	0.25±0.01*	0.33±0.01	0.33±0.01*
Eicosatrienoic, 20:3	1.74±0.04	1.52±0.03*	1.80±0.04	1.90±0.02*
Eicosatetraenoic (arachidonic), 20:4	5.47±0.13	5.00±0.04*	5.60±0.13	5.79±0.05*
Eicosapentaenoic, 20:5	1.53±0.09	1.22±0.03*	1.94±0.05*	1.33±0.02*
Docosadienoic, 22:2	0.98±0.02	0.86±0.02*	1.02±0.02	1.08±0.02*
Docosatrienoic, 22:3	1.15±0.05	0.93±0.02*	1.36±0.03*	0.86±0.02*
Docosatetraenoic, 22:4	2.85±0.07	2.47±0.04*	3.00±0.06	3.09±0.08*
Docosapentaenoic, 22:5	4.71±0.11	4.23±0.05*	5.54±0.16*	4.28±0.08*
Docosahexaenoic, 22:6	5.82±0.14	5.18±0.07*	6.44±0.06*	5.43±0.15*
Total content of fatty acids	100.00	100.00	100.00	100.00
Including saturated	19.81	22.20	19.56	24.27
monounsaturated	37.82	40.64	35.46	33.63
polyunsaturated	42.37	37.76	44.98	42.10
ω-3/ω-6	0.79	0.78	0.89	0.66

Table 5. Fatty acid composition of esterified cholesterol of the rabbits' liver in acute L-arginine-induced pancreatitis + feeding of flaxseed/sunflower oils, % (M ± m, n=5)

Fatty acids and their code	Rabbits' groups			
	К	Р	Р+flaxseed oil	Р+sunflower oil
Caprylic, 8:0	0.16±0.01	0.22±0.01*	0.18±0.01	0.24±0.01*
Caprynic, 10:0	0.20±0.01	0.25±0.01*	0.22±0.01*	0.27±0.01*
Lauric, 12:0	0.29±0.01	0.34±0.01*	0.32±0.01*	0.36±0.01*
Myristic, 14:0	0.52±0.02	0.62±0.02*	0.55±0.02	0.65±0.02*
Pentadecanic, 15:0	0.31±0.01	0.40±0.02*	0.34±0.01	0.40±0.01*
Palmitic, 16:0	8.46±0.22	9.69±0.21*	8.79±0.22	9.78±0.21*
Palmitoleic, 16:1	0.95±0.02	1.00±0.03	0.99±0.02	1.02±0.05
Stearinic, 18:0	8.82±0.22	9.78±0.11*	8.55±0.23	10.44±0.30*
Oleic, 18:1	29.08±0.62	31.85±0.53*	25.09±0.83*	28.40±0.51
Linoleic, 18:2	14.51±0.30	13.14±0.10*	15.05±0.37	15.17±0.18*
Linolenic, 18:3	6.48±0.14	5.87±0.07*	7.14±0.09*	5.03±0.11*
Arachinic, 20:0	0.34±0.01	0.40±0.01*	0.28±0.01*	0.45±0.02*
Eicosaenoic, 20:1	0.19±0.01	0.21±0.01*	0.20±0.01	0.23±0.01*
Eicosadienoic, 20:2	0.30±0.01	0.24±0.01*	0.32±0.01	0.33±0.01*
Eicosatrienoic, 20:3	1.95±0.05	1.64±0.04*	2.04±0.05	2.14±0.06*
Eicosatetraenoic (arachidonic), 20:4	7.07±0.10	6.59±0.06*	7.34±0.10	7.38±0.10*
Eicosapentaenoic, 20:5	1.85±0.06	1.48±0.05*	2.20±0.06*	1.20±0.07*
Docosadienoic, 22:2	0.95±0.02	0.82±0.02*	0.97±0.02	1.02±0.03*
Docosatrienoic, 22:3	1.30±0.06	0.99±0.03*	1.58±0.04*	1.14±0.05*
Docosatetraenoic, 22:4	3.21±0.07	2.85±0.04*	3.43±0.07*	3.43±0.07*
Docosapentaenoic, 22:5	6.13±0.17	5.42±0.07*	6.84±0.06*	5.08±0.13*
Docosahexaenoic, 22:6	6.93±0.14	6.19±0.08*	7.57±0.08*	5.82±0.13*
Total content of fatty acids	100.00	100.00	100.00	100.00
Including saturated	19.10	21.70	19.23	22.60
monounsaturated	30.23	33.07	26.29	29.65
polyunsaturated	50.67	45.23	54.48	47.75
ω-3/ω-6	0.81	0.79	0.87	0.62

Table 6. Fatty acid composition of skeletal muscles' esterified cholesterol of rabbits in acute L-arginine-induced pancreatitis + feeding flaxseed/sunflower oils, % (M ± m, n=5)

Fatty acids and their code	Rabbits' groups			
	К	Р	Р+flaxseed oil	Р+sunflower oil
Caprylic, 8:0	0.12±0.01	0.17±0.01*	0.13±0.01	0.18±0.01*
Caprynic, 10:0	0.18±0.01	0.24±0.01*	0.20±0.01	0.26±0.01*
Lauric, 12:0	0.29±0.01	0.36±0.01*	0.31±0.01	0.38±0.01*
Myristic, 14:0	0.51±0.02	0.62±0.02*	0.54±0.02	0.65±0.02*
Pentadecanic, 15:0	0.31±0.01	0.37±0.01*	0.33±0.01	0.37±0.01*
Palmitic, 16:0	10.53±0.43	12.03±0.08*	10.86±0.47	12.82±0.36*
Palmitoleic, 16:1	1.05±0.05	1.37±0.05*	1.14±0.06	1.41±0.07*
Stearinic, 18:0	12.51±0.51	15.01±0.45*	11.91±0.42	15.79±0.51*
Oleic, 18:1	37.58±0.58	37.12±0.62	34.77±0.73*	33.34±0.62*
Linoleic, 18:2	9.01±0.15	8.32±0.09*	9.33±0.15	9.37±0.10*
Linolenic, 18:3	4.86±0.09	4.29±0.08*	5.52±0.08*	3.92±0.07*
Arachinic, 20:0	0.29±0.01	0.37±0.02*	0.27±0.01	0.40±0.02*
Eicosaenoic, 20:1	0.20±0.01	0.21±0.01	0.18±0.01	0.19±0.01
Eicosadienoic, 20:2	0.36±0.01	0.28±0.01*	0.38±0.01	0.39±0.01*
Eicosatrienoic, 20:3	1.72±0.03	1.46±0.05*	1.81±0.04	1.93±0.02*
Eicosatetraenoic (arachidonic), 20:4	4.82±0.10	4.19±0.09*	4.97±0.10	5.09±0.06*
Eicosapentaenoic, 20:5	1.22±0.03	1.01±0.04*	1.46±0.04*	0.80±0.03*
Docosadienoic, 22:2	1.08±0.04	0.90±0.02*	1.15±0.05	1.25±0.05*
Docosatrienoic, 22:3	1.13±0.03	0.95±0.02*	1.31±0.03*	0.88±0.02*
Docosatetraenoic, 22:4	2.63±0.06	2.27±0.05*	2.73±0.07	2.90±0.05*
Docosapentaenoic, 22:5	4.35±0.09	3.83±0.07*	4.87±0.06*	3.42±0.09*
Docosahexaenoic, 22:6	5.25±0.11	4.62±0.09*	5.85±0.08*	4.24±0.05*
Total content of fatty acids	100.00	100.00	100.00	100.00
Including saturated	24.74	29.17	24.54	30.87
monounsaturated	38.83	38.70	36.10	34.94
polyunsaturated	36.43	32.13	39.36	34.19
ω-3/ω-6	0.86	0.84	0.93	0.63

Table 7. The content of bile acids, 25-OH vitamin D_3, testosterone, aldosterone and cortisol in the blood of rabbits depending on the concentration and fatty acid composition of esterified cholesterol (M ± m, n=5)

The studied indicator	Rabbits' groups			
	К	Р	Р+flaxseed oil	Р+sunflower oil
Esterified cholesterol and polyunsaturated fatty acids of the families ω-3 and ω-6				
Esterified cholesterol, g/l	0.83±0.06	1.01±0.01*	0.79±0.06	1.05±0.01*
Fatty acids of ω-3 and ω-6 families, %	42.4±1.1	37.8±0.8*	45.2±1.2	40.66±1.2
including fatty acids of ω-3 family, %	18.6±0.5	16.5±0.5*	21.2±0.5*	16.78±0.4*
Cholesterol derivatives				
Taurocholic, g • 10^{-3}/l	0.42±0.03	0.33±0.01*	0.54±0.01*	0.29±0.01*
Glycocholic, g • 10^{-3}/l	0.57±0.02	0.45±0.02*	0.67±0.01*	0.40±0.01*
Glycodeoxycholic, g • 10^{-3}/l	0.23±0.01	0.16±0.01*	0.31±0.01*	0.14±0.01*
Cholic, g • 10^{-3}/l	0.20±0.01	0.14±0.01*	0.27±0.01*	0.12±0.01*
Deoxycholic, g • 10^{-3}/l	0.74±0.03	0.59±0.01*	0.89±0.02*	0.54±0.02*
25-OH vitamin D_3, g • 10^{-6}/l	3.88±0.18	3.21±0.04*	4.62±0.05*	3.05±0.07*
Testosterone, g • 10^{-6}/l	2.75±0.11	2.86±0.10	3.19±0.04*	2.52±0.05*
Aldosterone, g • 10^{-9}/l	988.3±35.7	1040.3±25.7	1172.5±21.0*	907.4±24.4*
Cortisol, g • 10^{-6}/l	40.4±1.9	43.6±2.0	51.7±2.1*	36.5±0.8*

The above table also shows that the concentration of taurocholic, glycocholic, glycodeoxycholic, cholic and deoxycholic acids in the serum, 25-OH vitamin D_3, testosterone, aldosterone and cortisol in the blood plasma of rabbits increases with normal content of rich in polyunsaturated fatty acids of the families ω-6 and, especially, ω-3 esterified cholesterol in blood plasma.

Predominant esterification of blood plasma, liver and skeletal muscle of rabbits with polyunsaturated fatty acids in acute L-arginine-induced pancreatitis, corrected by fed flaxseed oil, may indicate a decrease in its crystallinity and improvement of interstitial transport (Drogomyretska et al., 2010). In the liver, skin, adrenal glands and gonads it is converted into appropriate derivatives: bile acids, vitamin D_3, estrogens, androgens and corticosteroles (Chen et al., 1999).

CONCLUSIONS

The positive corrective effect of fed flaxseed oil on the condition of the pancreas in acute L-arginine-induced pancreatitis was shown, the development of which was assessed by the number of necrotized acinar epitheliocytes in the head and tail of the pancreas and the activity of lipase and α-amylase in blood plasma. Feeding sunflower oil does not show a similar corrective effect.

The normalizing effect of fed flaxseed oil on the state of the antioxidant defense system in rabbits in acute L-arginine-induced pancreatitis, on the content of thiobarbituric acid-positive products and the activity of superoxide dismutase, catalase and glutathione peroxidases in blood. Feeding sunflower oil leads to a deterioration of the oxidative-prooxidant balance.

The ability of fed flaxseed oil to prevent disorders of content of non-esterified and esterified cholesterol in blood plasma, liver and skeletal muscle of rabbits in acute L-arginine-induced pancreatitis has been established. Feeding sunflower oil under the above conditions impairs the lipid composition of rabbit tissues.

The positive effect of fed flaxseed oil on the ratio of anti-inflammatory polyunsaturated fatty acids of the ω-3 family to proinflammatory polyunsaturated fatty acids of the ω-6 family in the fatty acid spectrum of esterified cholesterol in blood plasma, liver and skeletal muscle of rabbits in acute L-arginine-induced pancreatitis. The result of the fed sunflower oil action on the studied indicators is quite the opposite.

In acute L-arginine-induced pancreatitis, feeding flaxseed oil stimulates the conversion of cholesterol to bile acids, 25-OH-vitamin D_3, testosterone, aldosterone and cortisol in rabbits. The stimulating ability of fed sunflower oil in this regard is less pronounced.

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