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EXPERIMENTAL SUBSTANTIATION EFFICIENCY OF APPLICATION OF THE AMARANTH SEEDS OIL AT COMPLICATIONS INVOKED BY ISONIAZID

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Abstract. In an experimental research found that amaranth seeds press oil in dose of 600 mg/kg of the Isoniazid intoxication (in dose of 542 mg/kg during 6 days) prevents death of animals, reduces considerably an expressiveness of central and peripheral nervous system pathological changes, promotes normalization of digestive function and animals clinical state, lowers cytolysis syndrome manifestation degree promoting full normalization of aminotransferases activity on the 14th day. By means of biomicroscopy, using the original method of rats small intestine mesentery microvascular monitoring in an abdominal cavity, the peculiarities of microcirculatory processes disturbances invoked by high doses of Isoniazid, are revealed for the first time and the possibility of it correction via amaranth seeds oil.
Keywords: amaranth seeds oil, isoniazid, intoxication, hepatoprotective activity, microcirculation.

Introduction.

In modern medicine the Isoniazid remains one of the main preparations applied at tuberculosis chemotherapy. It is used as monotherapy at primary and secondary chemoprophylaxis of the infected persons contacted on tuberculosis as well as a part of the combined schemes of tuberculosis chemotherapy. Along with high tuberculostatic activity Isoniazid possesses the high specific organotropic toxicity also [1, 2]. Drug-induced liver damages (DILD) and polyneuropathy are commonly encountered complications of Isoniazid chemotherapy, whereas cardiovascular system reactions and gastrointestinal tract dysfunctions are observed rare.

System character of the undesirable adverse reactions developing at Isoniazid chemotherapy dictates need of simultaneous and prolonged use of several preparations for complications correction and thus increases the toxic load of the liver. It causes the necessity to search new remedies with wide range of pharmacological activity, influencing directly on the main links of tuberculosis pharmacotherapy complications pathogenesis, and the DILP in the first place. In particular, the creation of new medicines containing natural origin substances remains actual.

Amaranth family plants (Amaranthaceae) are widely cultivated in the Central Chernozem region. They are considered to be one of the possible sources of biologically active substances which seeds contain the fat oil with wide range of pharmacological activity provided by it biologically active agents (phospholipids, phytosterols, carotinoids, tokoferol and squalene) [3, 4].

Considering that polyneuropathies, DILD, cardiovascular and gastrointestinal tract adverse reactions dominate in the frequency and severity of Isoniazid chemotherapy treatment complications [5, 6], it will be reasonable to study the amaranth seeds oil application due to it antioxidant, gastric, hepatic and cardio protective action founded for correction of chemotherapy complications pathogenically.

As follows from the above, the objective of this research **i**s the possibility of amaranth seeds press oil application for prophylaxis and treatment of the Isoniazid induced complications.

Materials and methods.

This research is executed in the department of pharmacology of the Voronezh state university with active support of department of clinical pharmacology of N. N. Burdenko Voronezh state medical university according to the recommendations stated in the Guide for medicines preclinical experimentation [7], with observance of bioethics principles, methodical approaches to experiments quality control and monitoring of laboratory animals health [8]. Experiments are approved by the ethical committee on biomedical researches examination of the Voronezh state university (protocol № 42-01 of 23.09.2015).

Researches were performed on 305 puberal conventional nonlinear white male rats at the age of 3 months with body weight of 205,0±9,2 g. Animals were accommodated in plastic cages at natural light regimen. Feeding was carried out by the standard certified combined feed in accordance to the existing rules at free access to water and food. Euthanasia of animals was performed according to the requirements of "Iinternational recommendations on medicobiological researches conducted on animals". During all researches conduction we followed the principle of pair analogs in animal selection for sexual, age and body weight parameters.

The unrefined oil made of germs and seed coats of amaranth we used as object of this research. It was produced industrially (JSC Russkaya Oliva, Russia) by method of cold passage pressing (in further – the amaranth seeds press oil).

Calculation of the amaranth seeds press oil doses was carried out individually for each animal according to their body weight (mg/kg of animal mass) taking into consideration the specific density of oil, which was equal to 0,936 g/sm^3. The optimum therapeutic dose was defined according to literature data with regard to own results of it hepatoprotective activity screening investigations [9].

To realize the proper intoxication model the Isoniazid was introduced into rats stomach (control group) in the form of 1% of starched slime in dose of 542 mg/kg during 6 days in the morning before the main feeding [7, 10]. Oil of amaranth seeds was administrated in dose of 600 mg/kg (with 50 mg/kg phospholipids content) into stomach for 6 days with the prophylaxis purpose in 1 hour before the Isoniazid introduction, and with the curative aim in the same dose in 24 hours after the last introduction of the Isoniazid once per day within 3 days (main groups). According to the methodical recommendations on phospholipids drugs hepatoprotective activity studying [7] as drug of comparison the "Essentiale N" hepatoprotector (C. A. Sanofi-Aventis, further - EFL) was chosen. It contains the essential phospholipids of soybeans and is included into the Standard of medical care for tuberculosis patients. It was introduced in dose of 80 mg/kg (group of comparison) according to the schemes similar for amaranth seeds oil. Healthy animals were taken as an intact group. Introduction of the studied substances into stomach was realized by metal nontraumatic gastric tube.

In 1 hour and 24 hours after the Isoniazid single dosing, and also on 3rd, 7th, 10th and 14th day the assessment of animals psychoemotional condition, it heart functional activity, microcirculatory processes intensity, biochemical and histochemical researches were produced.

The psychoemotional condition of animals was characterized by creation of heuristic model of animals rescue task solution search from emotional and physical extreme situation by Yu. N. Chernov et al. in 1989 [11] in modification (Russian Federation Patent № 2506649) - the Heuristic Decisions test [12]. Modeling of stressful situation was reached by animals exposition into the cylinder with cold water (T=11°C). Time spent on the decision making (TD) and task execution time (TE) (to leave the cylinder by means of the offered survival equipment - lath and rope) were measured. According to the technique we calculated the percentage probability of task solution (PTS), Index of psychoemotional (IPI) and locomotor (ILI) influence.

Influence on heart functional activity was studied by electrocardiogram (ECG) analysis. The ECG record at non-anesthetized fixed animals was taken at 20 mv strengthening and 50 mm/sec speed of tape movement in the second standard lead on the EK1T-04 AXION electrocardiograph (Russia).

Impact of the amaranth seeds oil on parametres of liver functional activity was estimated by extent of biochemical, histochemical and patologomorfological indexes normalization. Blood and internals (liver) sampling for research was executed in the morning after animals deprivation right after rats were decapitated under the chloroformic anesthesia.

Biochemical research of blood serum has been executed on the biochemical analyzer of open type (Furuno 270). It consists of measuring of liver enzymes activity - alaninaminotranspherase (ALAT) and aspartate aminotransferase (AsAT) - by kinetic method (the UF-test according to the IFCC method) with use of standard diagnostic sets "Fluitest GPT", E/l; total bilirubin content estimation (mcmol/l) by photometric method with use of DiaS diagnostic sets; general cholesterol concentration measurment (mM/l) by enzymatic colorimetric method with use of the Fluitest CHOL diagnostic set. Definition of prothrombin time (sec.) was performed with use of the Diagem-P diagnostic set. The activity of lipid peroxidation processes (LP) we estimated by secondary lipoperoksidative products quantity reacting with thiobarbituric acid (TBA-active products, nM/l).

The liver histomorphology was studied in samples staining by hematoxylin and eosine. The histochemical picture was investigated by Oil Red O staining for lipids identification. For semi-quantitative assessment of the lipid amount the five-point scale was used [7].

To estimate the microcirculatory bed reaction in small intestine mesentery we anesthetized rats by intraperitoneal introduction of glucochloral fresh solution (40 mg/kg) and urethane (6 mg/kg) using passing light biomicroscopy according to the technique offered by A.M. Chernukh et al. in 1975 in modification (Russian Federation Patent № 2555136) by the unit (Russian Federation Patent № 152550) created on the basis of BIOMED-1 microscope with use of the offered way of abdominal cavity microvessels monitoring (Russian Federation Patent № 2506649).

After animals anesthesia we performed the median laparotomy [13] and defined the presence of hemorrhages in mesentery "windows" (site between two large veins) [14] and measured the square of it areas with palettes with coordinate grid [15].

We registered the biomicroscopy violations in microvascular region: [14, 16]:

1) intravascular violations such as rheological disoders;

2) vascular violations such as changes of microvascular walls permeability, blood corpuscles diapedesis and microhemorrhages. For the semi-quantitative characteristic of microcirculatory bed condition we carried out the blood-flow assessment by mark scale [17].

Video registration of small intestine mesentery microcirculatory bed research in different states was realized by means of digital video camera for Levenhuk 8 Mpixels microscope installed on the eyepiece and connected with the personal computer. The instrument magnification was 40 and 80 times. Average values of diameter (mcm) of all blood vessels in target mesentery segment were defined. Average quantity of capillaries in the area of investigated segment in 1 mm^2, average diameter and length of capillaries (mcm), surface area of capillaries as cylinder surface with related crossection (1) and space occupied by capillaries in the studied segment (2) were calculated [16]:

$$S\,к = \pi \times D \times L \qquad (1),$$

where Sk is the surface area of capillaries, mm^2;

D is average diameter of capillaries, mcm;

L is average length of capillaries, mcm;

$$Sc = Sк \times Nк \qquad (2),$$

where Sc is the space occupied by capillaries in the studied segment, mm^2;

Nk is average quantity of capillaries in the studied segment, units.

In pictures of microscopic and histochemical sections of liver made by the computer ToupView program we determinated the linear sizes of cells and nucleuses of hepatocytes (mcm), apoptosis index (as ratio of number of cells apoptosis state to total number of cells) and percent of hepatocytes necrosis (as ratio of the space occupied by hepatocytes with necrosis signs to the total studied liver parenchyma area).

Statistical analysis was produced with use of parametrical and nonparametric methods [18] by STATISTICA 8.0 program set. For quantitative indices we calculated the descriptive average, standard deviation and error of mean.

Results.

It is established that amaranth seeds oil introduction applied to avoid the Isoniazid induced complications prevents convulsions in control group of animals that lasted 40 - 150 minutes in 100% of observed cases as well as death of rats in 33% of cases during 3 hours after the Isoniazid single introduction in dose of 542 mg/kg. This result exceed the efficacy of comparison remedy. We observed short convulsions without lethal outcome after it application in 16% of rats.

After use of the amaranth seeds oil on the background of sixfold Isoniazid introduction the registered indexes of animals clinical condition corresponded to norm parameters for adult healthy animals on 7th day of observation. Whereas in the survived animals of the control group the clinical picture of intoxication during 10 days of research was characterized by heart functional activity violations (reduction of QT interval length according to the ECG analysis, $p<0,05$) and gastrointestinal tract disorders (abdominal distention and reduction of food consumption on 58%-90%, $p<0,01$).

The probability of task solution in the Heuristic Decisions test on the 7th day of research exceeded the control group level as well as level of comparison preparation group on 27,7% and 14,7%, respectively. This effect was observed on the background of decrease of the time spent on the decision making and task execution time in 1,7 times and 1,9 times, respectively ($p<0,01$). General time of task execution on 10^{th} day of experiment was 3,7 times smaller than in control group ($p<0,01$). According to calculations the IPI was equal to 0,99 whereas ILI was equal to 0,59 that in general argues about normalization of psychomotor and physical activity of animals as well as their general clinical state (table 1).

Table 1

Effect of amaranth seed oil pressing to change the psychoemotional and locomotor responses in rats with isoniazid intoxication

Name	Indicators					
	TD, sec.	TE, sec.	TD + TE, sec.	PTS, %	IPI	ILI
Original	7,43±1,63	2,38±0,22	9,20±2,42	100	–	–
24 hours after the Isoniazid single dosing						
Control	4,20±1,56	3,02±1,28	7,22±2,46	100	–	–
EFL	4,18±1,83	2,38±0,89	6,56±2,72	100	0,99	0,88
AO	2,47±1,08**	1, 98 ±0,54	4,45±0,54**	100	0,85	0,80
3rd day of experiment						
Control	5,59±2,85	4,36±2,19	9,95±5,04	100	–	–
EFL	1,89±1,32*	2,25±0,54	4,14±1,86**	100	0,71	0,69
AO	2,0±1,40**	1,92±0,66	3,92±1,21**	100	0,72	0,64
7th day of experiment						
Control	17,20±3,40**	12,97±2,25*	30,17±5,65*	57	–	–
EFL	22,60±3,4**	11,4±2,59*	34,0±5,99*	71,4	1,01	0,89
AO	10,30±2,27++●	6,75±2,36**++	17,05±4,0*++●●	85,7	0,99	0,59
10th day of experiment						
Control	10,40±4,35	10,60±2,14*	21,0±6,49*	100	–	–
EFL	3,50±1,25**	4,20±0,41*+	7,70±1,66++	100	0,61	0,51
AO	2,32±0,52*++	3,40±2,07++	5,72±2,59++	100	0,54	0,44
14th day of experiment						
Control	4,72±0,78	2,64±0,55	7,36±1,33	83,4	–	–
EFL	3,13±0,94**	2,26±1,17	5,39±2,11	100	0,80	0,85
AO	2,38±0,89*++	2,25±0,89	4,63±1,77**	100	0,74	0,85

Note: EFL – essential phospholipids preparation; AO – amaranth seeds oil; Student's test: * - p<0,05; ** - p<0,01 – as compared to original; + - p<0,05; ++ - p<0,01 – as compared to control group; • - p<0,05; •• - p<0,01 – as compared to EFL; TD – time spent on the decision making; TE – task execution time; PTS – probability of task solution, %; IPI – Index of psychoemotional; ILI – locomotor influence.

Results of biochemical blood serum analysis provided in the table 2 argue that sixfold Isoniazid introduction, as well as single reception of its high dose, was followed by injury of liver. Statistically significant changes of level of the studied biochemical parameters show this lesions obviously just as a development of liver diffuse fatty degeneration and reliable increase of prothrombin time.

Introduction of the amaranth seeds oil during the first hours of intoxication prevented the development of hepatocytes cytolysis syndrome. It was proved by the reduction of ALAT activity in 1 hour after the Isoniazid introduction in comparison with control and preparation of comparison groups on 33% and 26%, respectively (p <0,05) with the subsequent return to norm in 24 hours when activity of ALAT and ASAT was twice higher than rates of control group animals which for 10 days of observation demonstrated sharp decrease of the aminotransferases activity.

At sixfold Isoniazid introduction the activity of ASAT was normalized on 10th day whereas ALAT activity - on 14th day of observation. These indexes did not return to initial level in control group at the same period. The amaranth seeds press oil influence on these enzymes was comparable to action of comparison preparation.

Along with effects described before the amaranth seeds press oil promoted the normalization of lipid metabolism lowering the blood serum general cholesterol concentration on 22-35% (table 2) and reduced prothrombin time throughout the entire period of observation, on the 7th day the maximum was registrated on 34,2% , p <0,05. It also prevented the lipoperoxidation processes activation what was confirmed by reduction of rate of TBA-active products in blood serum within the first days of intoxication on 15-19% (p <0,05) compared with control with the subsequent normalization on 3rd day of research period.

Table 2

Change of biochemical indexes in rat blood serum under introduction of amaranth seed oil press for prophylaxis of the Isoniazid intoxication

Name	ALAT, E/l	AsAT, E/l	Total bilirubin, mcmol/l	General cholesterol, mM/l
Intact group	55,60±7,75	144,91±21,67	2,56±0,8	1,59±0,13
1 hour after the Isoniazid single dosing				
Control	108,57±11,6*	163,46±13,03	2,32±0,28	1,64±0,62
EFL	98,94±7,44*	174,52±12,8*	2,67±2,36	1,62±0,12
AO	72,64±2,73*+●	207,98±21,5*+	1,79±0,1+	1,80±0,15
24 hours after the Isoniazid single dosing				
Control	23,49±6,92*	81,92±25,84*	0,98±0,35	3,13±0,80*
EFL	40,90±4,23*+	161,10±17,6+	2,02±0,23+	1,82±0,36++
AO	49,91±4,92+	167,24±7,80++	1,27±1,30	2,03±0,24++
3rd day of experiment				
Control	21,72±6,68*	65,80±6,92*	1,87±0,26	2,72±0,38*
EFL	28,19±4,09*	95,66±12,32*+	1,80±0,91	2,65±0,53**
AO	31,02±10,53*	134,66±28,62+●	2,74±0,18+●	2,12±0,21*+
7th day of experiment				
Control	16,55±6,55*	50,90±7,73*	2,48±0,33	2,24±0,10*
EFL	22,25±2,07*	139,50±22,3+	2,43±1,30	2,56±0,45**
AO	20,54±3,28*	90,41±19,50*+	2,30±1,11	2,04±0,17*
10th day of experiment				
Control	18,52±5,75*	71,68±28,66*	1,70±0,004**	1,45±0,10
EFL	25,04±8,62*	116,06±17,6+	1,34±0,69	1,49±0,33
AO	28,78±5,63**	147,84±23,7+	1,98±1,04	1,41±0,07**
14th day of experiment				
Control	43,04±3,91*	202,36±39,3**	1,82±0,32	1,86±0,26
EFL	46,51±8,55	127,63±8,58+	2,55±0,1*+	1,92±0,35
AO	53,71±1,58++	147,16±26,57++	2,50±0,35*+	1,64±0,15

Note: EFL – essential phospholipids preparation; AO – amaranth seeds oil; Newman's-Keylsa test with Bonferroni correction: * - p<0,05; ** - p<0,01 – as compared to intact group; + - p<0,05; ++ - p<0,01 – as compared to control group; • - p<0,05; •• - p<0,01 – as compared to EFL.

Pathoanatomical researches in control group revealed the significant increase of stomach size connected with its overflow by undigested food masses since 3rd day of experiment. Whereas in the experimental group this parameter corresponded to physiological standard (fig. 1) that is probably can be explained by pro-kinetic action typical for vegetable oils. The mass coefficient of liver on the 7th day of intoxication was lower than in control group on 18% (p <0,01) and corresponded to intact animals value on 10th day of observation.

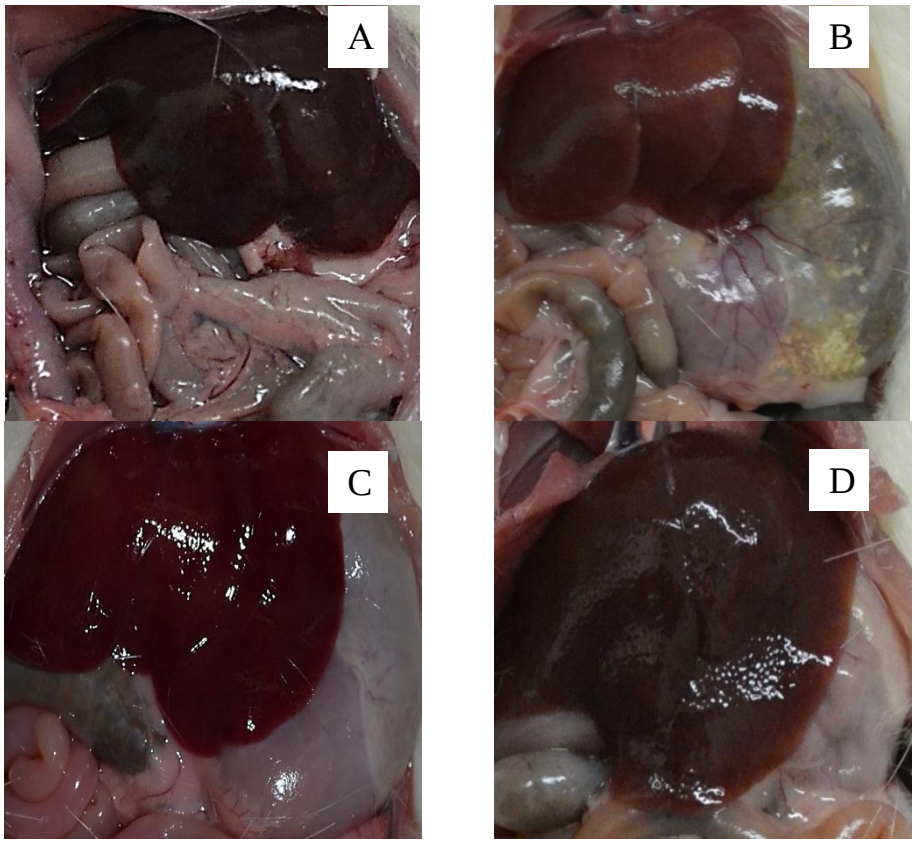

Figure 1. The stomach of rats under introduction of amaranth seed oil press for prophylaxis under the conditions of the Isoniazid intoxication, 3rd day of experiment.

A – intact group: stomach the normal form, the contents of the stomach uniform; B – control group: the significant increase of stomach size connected with its overflow by undigested food masses; C – essential phospholipids preparation: ventricle slightly swollen; D – amaranth seeds oil: stomach the normal form.

The hemorrhages registered in "windows" of mesentery of animals in control group (including rats which dead during the first hours after intoxication) were absent (fig. 2).

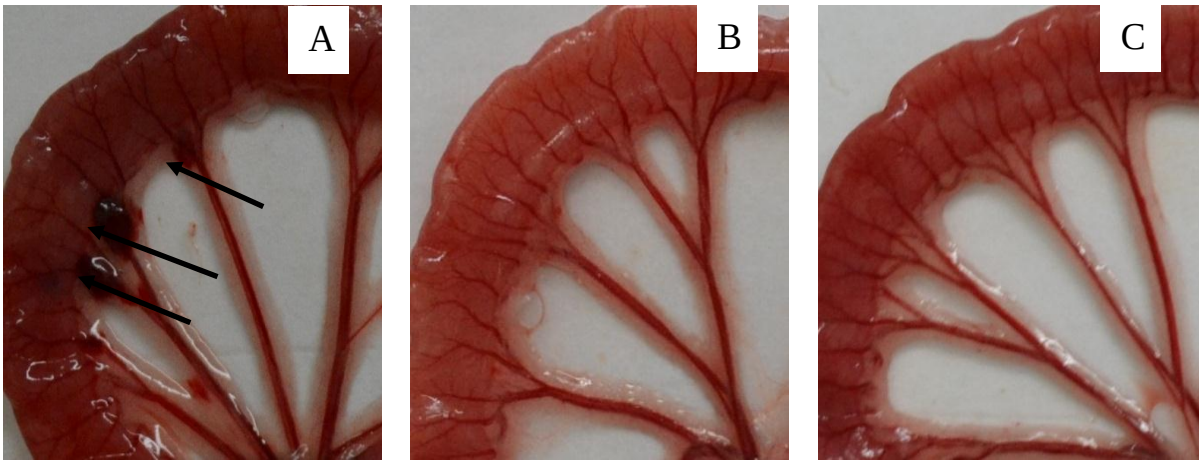

Figure 2. Rats small intestine mesentery under introduction of amaranth seed oil press for prophylaxis under the conditions of the Isoniazid intoxication, 1 hours after the Isoniazid single dosing: A – control group; B – п essential phospholipids preparation; C – amaranth seeds oil; arrows indicate hemorrhage.

Biomicroscopy of small intestine mesentery blood vessels performed in the passing light shown that control animals had the change of microcirculation vessels diameter, intravascular disorders followed by blood cells aggregation, signs of generalized sladge-syndrome and stasis and also vascular wall violations which morphological manifestation were blood cells emigration and hemorrhages with the diameter of 1,9-

9,9 ×10 $^{-3}$ mm^2 at natural blood filling of microcirculation vessels and preservation of vascular tone throughout the entire period of observation. Use of amaranth seeds oil in regimen of prophylaxis and treatment prevented hemorrhages appearance and blood corpuscles emigration (fig. 3).

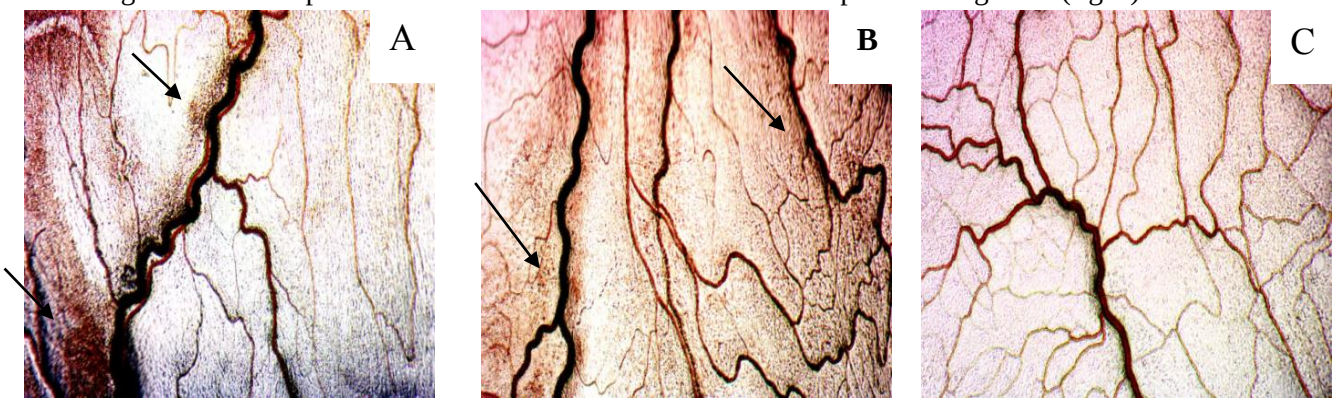

Figure 3. The microcirculation bed of the small intestine mesentery under introduction of amaranth seed oil press for prophylaxis under the conditions of the Isoniazid intoxication, 1 hours after the Isoniazid single dosing. Biomicroscopy, ×40: A – control group; B – essential phospholipids preparation; C – amaranth seeds oil.

Introduction of the amaranth seeds oil promoted blood-flow recovery, thus the assessment of its intensity change in mesentery microcirculatory vessels according to mark scale on the 7th day of observation was 20,0±0,0 points that corresponded to data of intact group animals, against 17,0±1,25 points in control group.

The morphometry has revealed the increase of venules diameter on 13-19% concerning the intact rats ($p<0,05$) as well as diameter of capillaries on 29,5% ($p<0,01$) and 35,3% ($p<0,05$) on the 10th day maximally concerning the intact and control rats, respectively (table 3).

Table 3

Change of diameter of the mesentery microvessels in rats under introduction of amaranth seed oil press for prophylaxis under the conditions of the Isoniazid intoxication

Name	Diameter of blood vessels, mcm				
	Arterioles	Precapillary	Capillaries	Postcapillary venule	Venules
Intact	41,7±1,5	12,8±1,2	7,1±1,0	19,5±0,7	44,0±1,8
1 hours after the Isoniazid single dosing					
Control	40,1±1,2	14,5±0,5	6,4±1,2	25,4±1,9*	50,1±1,2*
EFL	41,4±1,4	12,4±1,0++	7,2±0,4	20,6±1,5++	42,6±1,0+
AO	41,6±2,0	12,8±0,8++	7,4±0,2	19,7±1,1++	44,2±1,4+
24 hours after the Isoniazid single dosing					
Control	37,8±1,4	11,9±1,7	8,2±0,3	20,7±1,9	46,5±2,4
EFL	41,3±0,9++	15,8±0,4**++	6,25±1,1++	19,1±0,4	49,5±0,9*
AO	41,1±1,4++	14,7±0,2++	8,2±1,0	20,1±0,9	52,4±0,1*++
7th day of experiment					
Control	59,2±1,5*	13,8±1,2	6,5±0,9	27,5±1,4*	55,0±5,9**
EFL	46,8±1,9**+	12,7±1,0	7,0±0,5	20,2±1,0+	44,2±0,9++
AO	42,1±1,8+	13,0±1,2	8,4±0,3++	19,4±0,8+	52,4±0,4*
10th day of experiment					
Control	54,5±2,7*	10,7±1,3	6,8±0,6	20,8±0,6	45,2±1,4
EFL	48,1±2,4**++	12,9±1,2	6,5±0,6	20,0±0,6	46,4±1,8
AO	42,3±1,2+	12,9±1,0	9,2±0,4**+	19,4±0,9	51,2±0,8*+
14th day of experiment					
Control	38,0±2,2	13,3±1,1	6,2±1,25	20,4±1,1	45,0±1,1
EFL	38,1±1,8	13,7±0,4	7,1±0,6	21,2±1,0	47,8±2,6
AO	42,1±1,2++	13,4±2,5	8,3±0,8	20,8±0,4	46,2±1,4

Note: EFL – essential phospholipids preparation; AO – amaranth seeds oil; Student's test: * - $p<0,05$; ** - $p<0,01$ – as compared to intact group; + - $p<0,05$; ++ - $p<0,01$ – as compared to control group.

Thus the average quantity of capillaries per 1 mm^2 of mesentery area was 2,4 times higher than in control (p<0,01), and the space occupied by capillaries exceeded the intact level in 1,45 times (p<0,01) (table 4).

Table 4

Change of quantity of capillaries and surface area of capillaries under introduction of amaranth seed oil press for prophylaxis under the conditions of the Isoniazid intoxication

Name	Day of experiment				
	1 hours	24 hours	7th day	10th day	14th day
quantity of capillaries in the area of investigated segment in 1 mm^2					
Intact	80,0±11,2				
Control	62,5±14,4	125,0±20,1**	43,7±23,9**	35,0±13,69**	62,5±13,6
EFL	75,0±17,7	72,5±15,0++	50,0±17,7	60,0±13,7	65,0±13,7
AO	85,0±22,4	74,0±13,4++	80,0±12,5	83,3±12,9++	106,2±12,5**++●●
Surface area of capillaries of investigated segment in 1 mm^2, mm^2					
Intact	0,36±0,08				
Control	0,28±0,07	0,61±0,12**	0,23±0,01**	0,21±0,04**	0,26±0,08
EFL	0,37±0,14	0,30±0,09++	0,26±0,12	0,27±0,10	0,30±0,11
AO	0,36±0,03	0,37±0,08++	0,41±0,15	0,53±0,16**+●●	0,54±0,025**+●●

Note: EFL – essential phospholipids preparation; AO – amaranth seeds oil; Student's test: ** - p<0,01 – as compared to intact group; + - p<0,05; ++ - p<0,01 – as compared to control group; •• - p<0,01 – as compared to EFL.

Histomorphological investigation established that in parallel with the biochemical data and microcirculatory processes improvement the amaranth seeds oil provided recovery of liver histoarchitectonics, prevented dystrophic and necrobiotic processes after Isoniazid single dosing and reduced it degree after it sixfold introduction, expressively. Apoptosis index on the 14th day of research was 0,22±0,11 against 0,41±0,62 in control. Signs of microcirculatory processes violation such as increase of vascular permeability, stratification of vein wall, expansion of the interlobular veins, observed in control group, were absent after oil introduction. In histochemical picture of liver in control group of animals the diffuse fatty degeneration has been observed. In experimental group hepatocytes with fatty drops have been located mainly at periphery of hepatic tubules. It should be noted that efficacy of amaranth seeds press oil exceeded the EFL preparation, according to the histochemical researches.

Use of amaranth seeds oil for treatment on 10th day of observation leaded to normalization of clinical and psychoemotional state, ALAT, ASAT activity and total bilirubin concentration in blood serum of animals with Isoniazid induced heavy intoxication whereas in control group this indexes did not return to the initial rate after 14 days of experiment (table 5). Thus the mass coefficient of liver was below the control level on 16,7% (p <0,01) that complies with intact animals rate. It is corresponded to results of histochemical researches and probably is caused by the reduction of manifestation degree of liver fatty degeneration. Besides, the amaranth seeds press oil introduction was accompanied by reduce of general cholesterol concentration below the intact group level on 14,7% (p <0,01).

Table 5

Change of biochemical indexes in rat blood serum under introduction of amaranth seed oil press for treatment under the conditions of the Isoniazid intoxication

Name	ALAT, E/l	AsAT, E/l	Total bilirubin, mcmol/l	General cholesterol, mM/l
Intact group	55,60±7,75	144,91±21,67	2,56±0,8	1,59±0,13
10th day of experiment				
Control	18,52±5,75*	71,68±28,66*	1,70±0,004**	1,45±0,10
EFL	25,17±4,74*	155,10±56,32++	7,49±1,57*+	1,51±0,04
AO	47,17±8,29+●●	152,74±24,25+	2,50±1,25●●	1,33±0,14**
14th day of experiment				
Control	43,04±3,91*	202,36±39,31**	1,82±0,32	1,86±0,26
EFL	44,72±2,57	184,31±33,94	4,33±1,05**+	1,72±0,06
AO	58,25±2,0+●	149,66±30,65++	2,55±1,1	1,89±0,28

Note: EFL – essential phospholipids preparation; AO – amaranth seeds oil; Newman's-Keylsa test with Bonferroni correction: * - p<0,05; ** - p<0,01 – as compared to intact group; + - p<0,05; ++ - p<0,01 – as compared to control group; • - p<0,05; •• - p<0,01 – as compared to EFL.

On the 14th day of experiment the prothrombin time was below the control rate on 12,8% ($p<0,05$). Reduction of TBA-active products concentration revealed in blood serum on 16,1% ($p<0,05$) argued about lipid peroxidation processes decrease.

Use of amaranth seeds press oil for treatment promoted blood-flow recovery and prevented development of destructive violations in great and microcirculatory vessels of small intestine mesentery, observed in the control group. Since 10th day of experiment, the morphometry has revealed an increase of venules diameter concerning intact and control groups on 16,0% and 12,8% respectively ($p <0,05$). On the 14th day the capillary diameter exceeded rate of intact and control groups (on 35,2%; $p <0,01$ and 54,8%; $p <0,01$), respectively) as well as diameter of venules (on 12,7% and 10,2% respectively, $p <0,05$). The vasodilation was accompanied by filling of reserve capillaries (quantity of capillaries per 1 mm^2 exceeded indexes of intact and control groups on 32,7% and 69,9% respectively, $p <0,05$) and increase of space occupied by this vessels in 1,3 times ($p <0,01$).

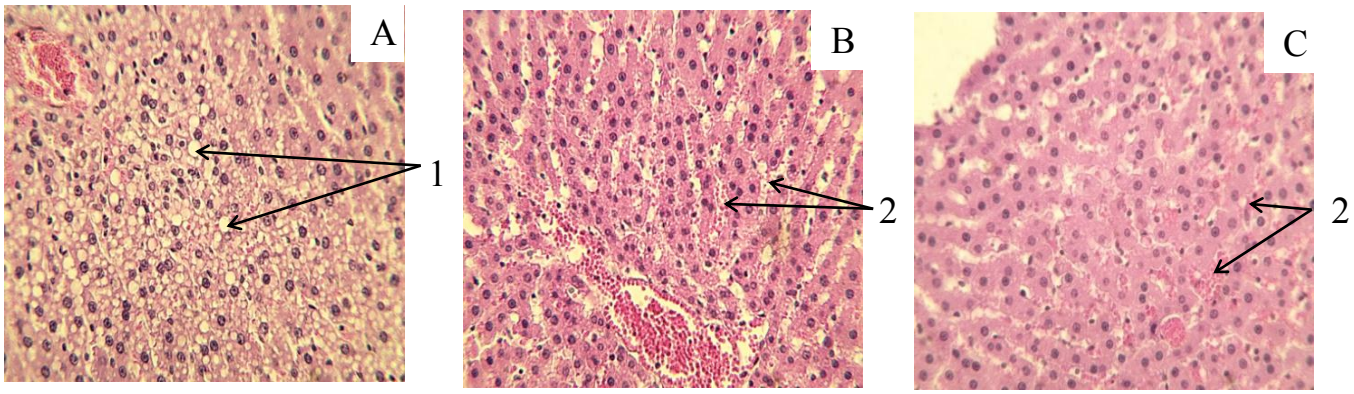

Figure 4. The histomorphological picture of liver under introduction of amaranth seed oil press for treatment under the conditions of the Isoniazid intoxication, 10th day of experiment, hematoxylin and eosine, ×400: A – control group: fatty degeneration (1); B – essential phospholipids preparation; C – amaranth seeds oil: increase of blood filling of vessels (2).

At the same time, the amaranth seeds oil provided liver histoarkhitektoniks recovery and reduced necrobiotic processes expressiveness on 10th day of observation, along with biochemical and microcirculatory processes normalization (fig. 4). The rate of apoptosis index for the 14th day of research was 0,24±0,07 against 0,41±0,62 in control group. The residual phenomena of fatty infiltration characterized the histochemical picture of liver after the amaranth seeds oil introduction unlike diffuse fatty degeneration, observed in control. The degree of fatty infiltration for the 10th days of observation was estimated as 0,7±0,2 points against 4,33±0,21 points in control.

Discussion.

Last years the data about large spectrum of pharmacological activity of the fatty oil containing in amaranth seeds and efficiency of its application in complex therapy of various diseases has appeared in a literature. According to available sources, the overwhelming majority of examinations are devoted to studying of properties of the oil produced by organic solvents extraction methods. Oil extraction is labour-consuming multiphase process that considerably raises received product price and does not exclude the residual dissolvent amount presence [4]. It is abundantly clear that this method can't be considered as optimal from the economic and technological expediency point of view. The solution of this problem is an application of the cold pressing method that provides conservation of biologically active substances in an invariable state that allows to prognosticate an absence of stimulating and cancerogenic activity and to reduce the price of oil considerably. Thus, the amaranth seeds oil, received by the cold pressing method, has a set of technological, economic and pharmakotoxicological advantages.

Employees of the Voronezh state university develop the original technology of the amaranth seeds oil production by the method of cold flow pressing [20], allowing to conserve biologically active substances in an invariable state and to reduce considerably the price of oil Safonova E.F. et alt. [21] offered the unified procedure of phospholipids separation process and analysis (2004). This method has allowed establishing of the quantitative and qualitative composition of the amaranth seeds oil phospholipid complex for the first time, separating a part of essential phospholipids (75 % of phospholipid complex mass, in average).

The model of the Isoniazid intoxication replicated during examination was characterized by the expressed disturbances in the central and peripheral nervous system, cardiovascular and hepatobiliary systems as well as in gastrointestinal

tract and microcirculatory bed. The amaranth seeds oil application for prophylaxis and treatment along with the death prevention levelled the disturbances of heart functional activity. Oil reduced expression of intoxication symptoms, promoting shortening of the period of registered indexes normalization in 1,5-2 times in animals general clinical state.

It is necessary to emphasize that the amaranth seeds press oil introduction reduced expression of intoxication symptoms revealed by heuristic model of animals rescue task solution search from emotional and physical extreme situation. Amaranth seeds press oil prevented reduction of psychomotor activity and disturbance of the skeletal muscles function, that were educed owing to damaging activity of the Isoniazid in CNS and peripheric innervation. It is possibly caused by improvement of animals general clinical state and liver detoxicative function. At the same time, application of the amaranth seeds press oil enhanced animals psychomotor response and motor activity that, in comparison with the EFL preparation action, was the distinctiveness of the oil action. The revealed changes are possibly bound with antioxidant and antihypoxant activity of the amaranth seeds press oil and are characterized by enriching of an aerobic metabolism parameters and adaptation potential magnitude, known from sportsmen clinical examinations [22].

Reduction of gastrointestinal tract disturbances manifestation degree is are possibly causeb by prokinetic activity typical for vegetable oils. This effect helps to overcome gastric distension and overflow with undigested alimentary masses as well as defecation absence in animals of the control group.

The data obtained at investigation concerning ability of the amaranth seeds press oil to reduce an expression of cytolysis syndrome arguing about damage of cytoplasmatic and mitochondrial hepatocytes membranes, allows to guess that one of the basic mechanisms of the amaranth seeds press oil hepatoprotective activity is membranoprotective action proved earlier on the acid hemolysis model [23].

Along with it, one of the probable mechanisms, allowing to explain the biochemical changes observed in blood serum on the background of intoxication, is reduction of lipid peroxidation processes activity. The amaranth seeds press oil exceeds the EFL preparation in efficacy of influence on this process that is possibly can be explained by presence of lipophilic antioxidants, such as tocopherols, lipochromes and squalene which potentiates the effect of phospholipids and raise the antioxidant activity [24].

Besides it necessary to take notice of biomicroscopy examinations of rats small intestine mesentery. These findings shown the ability of the amaranth seeds press oil to prevent the microcirculatory processes disturbances accompanied by intravascular aggregation of erythrocytes, stasis, destructive changes of microcirculation vessels walls, diapedesis and presence of hemorrhages observed at the Isoniazid intoxication.

The intravascular lesions and stasis observed in control by biomicroscopy on the background of the intoxication are possibly caused by changes of erythrocyte membrane functional properties owing to lipid metabolism displacement. This assumption is confirmed by literary data about changes of various phospholipid fractions in internal and external layers of erythrocytes membrane that are characterized by membrane permeability reduction and rigidity increasing under the conditions of liver toxic lesion [25, 26]. Proceeding from it, the preventive effect of the prophylactic and curating use of the amaranth seeds press oil on taped disturbances can be explained by change of membranes elasticity and thus their conformation properties at the expense of lipid metabolism processes restoration. The reduction of general cholesterol concentration in blood serum on 22,1-35,1% ($p<0,05$; $p<0,01$) confirms this guess. It corresponds to the reduction of liver fatty degeneration degree and prevalence.

As the microcirculatory part reacts as integrated system it is possible to estimate state of whole organism microcirculatory processes studying separate regions in a certain standard [27]. Ability of the amaranth seeds press oil to prevent disturbance of liver microcirculation and blood supply supports this opinion. This disturbances observed in control group are characterized by stratification of blood vessels walls, vascular permeability rising and interlobular veins dilation.

Thus reliable increase of the functioning capillaries quantity and space occupied by it in 1,3 - 1,5 times after amaranth seeds press oil application on the background of the Isoniazid intoxication attracts attention. This observations denotes an increase of circulatory bed capacity and improvement of oxygenation processes on the background of microcirculatory blood vessels vasodilation in venules anastomosis zone exactly. This facts agreed with effect of the amaranth seeds press oil local application on rats mesentery observed in healthy animals [28]. In general, it can be estimated as indirect mechanism of oil antyhypoxic activity.

Thus, as a result of the conducted researches the hepatoprotective effect of amaranth seeds press oil is

proved. This action is comparable to essential phospholipids preparation efficacy in expressiveness. The membrane protective, antioxidant and angioprotective properties of oil are revealed. It allows offering the amaranth seeds oil use for the first time for drug-induced liver damages correction in order to reduce the neuroautonomic, metabolic and microcirculatory violations observed at the Isoniazid chemotherapy.

Conclusions.

1. Introduction of amaranth seeds press oil in dose of 600 mg/kg for prophylaxis and treatment under the conditions of the Isoniazid intoxication (in dose of 542 mg/kg during 6 days) prevents developing of convulsions, violations of heart functional activity and death of animals, reduces considerably an expressiveness of CNS and peripheral nervous system pathological changes, promotes normalization of digestive function and animals clinical state on the 7th day in prophylaxis regimen and on the 10th day – in regimen of treatment.

2. At the drug-induced liver damage caused by the Isoniazid, the amaranth seeds press oil in dose of 600 mg/kg lowers cytolysis syndrome manifestation degree (ALAT activity is lower than control on 33% 1 hour after the Isoniazid introduction), promoting full normalization of aminotransferases activity on the 14th day (ASAT activity is lower than in control on 27%), provides reduction of necrobiotic processes intensity and liver histoarkhitektonik recovery, inhibits lipoperoxydation processes (reduces TBK-active products concentration on 15-19%), normalizes lipid metabolism (lowers the general cholesterol content on 22-35%). This effect is comparable to essential phospholipids preparation efficacy.

3. Introduction of amaranth seeds press oil in dose of 600 mg/kg prevents destructive violations of walls in great and microcirculatory blood vessels in rats' mesentery. It reduces expressively blood cells aggregation in microcirculative blood vessels and local stasis zones on the 7th day of the Isoniazid intoxication course after introduction in prophylaxis regimen and on the 10th day – after introduction in regimen of treatment. Oil causes reliable increase of space occupied by reserve capillaries (in 1,3-1,5 times) and causes it blood filling.

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