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EFFECT OF SELENIUM CHROME LIPID COMPLEX WITH *Chlorella vulgaris* Biej. ON THE CARBOHYDRATE AND LIPID METABOLISM IN EXPERIMENTAL TYPE 2 DIABETES

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Abstract
Through the incubation of single-celled alga Chlorella vulgaris Biej. in sodium selenite and chrome(III) chloride aquaculture, selenium chrome lipid substance has been obtained, separated and tested on the rats with experimental type 2 diabetes. Diabetes was induced in 2 stages: 1) high-calorie diet+sodium glutamate for 4 weeks; 2) intraperitoneal introduction of streptozotocin (65 mg/kg), preceded by nicotinamide (230 mg/kg). When introduced to the rats with experimental diabetes in 1 ml of 1% starch and water suspension, selenium (0.6mcg), chrome (1.05mcg) and lipid (0.5 mg) have been found to decrease intoxication and improve carbohydrate and lipid metabolism both in the liver and blood serum, thus proving efficacy for diabetes prevention and treatment.

Keywords: Chlorella vulgaris Beij., selenium chrome lipid complex, rats, type 2 diabetes, glucose, streptozotocin, nicotinamide.

Introduction. Diabetes being associated with concomitant diseases, complications and early disability, searching for the up-to-date prevention and treatment tactics is the issue of the day. As a spreading epidemic disease, type 2 obesity-aggravated diabetes is a global threat of the century and a challenging medical problem. According to scientific data [1], about 80–85 % patients with type 2 diabetes are inclined to excessive corpulence, therefore introducing a two-decades old term "diabesity" (combination of "diabetes" and "obesity"). Underlying type 2 diabetes are two crucial factors, or at least two genetic defects: insulin resistance and disturbed insulin secretion, the first causing insulin resistance and/or obesity, whereas the second - decreased β-cells' secretory activity or their sensitivity to high blood glucose content. The use of biologically active supplements (BAS) for the prevention of metabolic imbalance seems to be promising. BAS with selenium and essential metals are now widely used for the prevention of metabolic imbalance, but these are mostly physical mixtures of non-organic selenium compounds and metal salts, having low efficacy and causing side effects [2].

Both selenium and chrome are essential for the improvement of metabolism as well as for prevention and correction of a number of pathologies [3]. As regards chrome-and selenium-containing drugs, the intake of selenium-containing products does not fully meet the need of humans in selenium and many other microelements, much less their complex consumption.

Single-celled algae, containing biologically active substances which had been produced due to intracellular biosynthesis, are currently used as a source of organic compounds and microelements. They can absorb and accumulate exogenous microelements by including them into pigments, proteins and lipids [4]. Drugs of

chlorella *Chlorella vulgaris* proved to be the source of biologically available chlorophyll as well as of a series of vitamins, amino acids and fatty acid which possess antitoxic and antisclerotic characteristics [5]. We have already established optimal conditions for selenium and microelements' accumulation by chlorella cells in the aquaculture with an adequate medium for the production of nutriceutics [6]. Introduction of selenium chrome lipid substance to intact rats resulted in the increase of succinate dehydrogenase and cytochrome oxidase activity, as well as that of reduced glutathione. Besides, glutamate dehydrogenase way of glutamate formation has been found to become more intensive. The use of selenium chrome lipid complex contributed to better functioning of both antioxidant system components and energy exchange [2].

The above-said taken into account, our objective has been to study the effect of *Chlorella vulgaris* Beij-derived selenium chrome lipid complex on the metabolic processes in rats under experimental "diabesity", and to compare the effect of non-organic and organic chrome and selenium compounds on the rat metabolism under this pathology.

Materials and methods. BAS was obtained from unialgal *Chlorella vulgaris* Beij. CCAP- 211/11в, that had been grown in uptake conditions on the Fitzgerald medium, Zehnder and Gorham №11 modification at 22– 25℃, illumination 2500 lux, 16/8 h [7]. In line with previous findings [6], Na_2SeO_3 aqueous solution as calculated for Se^{4+} – 10,0 mg/dm^3 and $CrCl_3 \cdot 6H_2O$ aqueous solution with Cr^{3+} – 5,0 mg/dm^3 were added to the alga culture. Living cells' biomass was taken on the 7th cultivation day, the culture that had been grown in selenit- and chrome salts-free medium used as control.

Lipids were extracted from the alga biomass with chloroform-methanol mixture at 2:1 ratio by Folch method [8]: 20 weight particles of extraction mixture were added to 1 weight particle of wet biomass and left for 12 hours. Non-lipid extract admixtures were washed out with 1% KCl solution. The total number of lipids was determined by gravimetric method following the distillation of extraction mixture [9].

Selenium content in the lipid extract after its ignition with HNO_3 in hermetic weighing bottles at t=120°C for 2 hours was determined spectrophotometrically with o´- phenylene diamine, wave length 335 nm [10], whereas chrome content, after ignition with the mixture of HNO_3 and H_2SO_4 in hermetic weighing bottles, was determined spectrophotometrically with mordant S, wave length556 nm [11].

Subjected to research were 125 mongrel albino male rats (initial mass – 160 – 80 g). Keeping and manipulations were performed according to the regulations of "General Ethical Principles of Experiments on Animals", approved by the 1st National Congress on Bioethics (Kyiv, 2001) and the regulations of European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1985). The animals were kept in routine vivarium conditions. They were kept for 10 days in the experimental room for adaptation, then weighed and randomly divided into 7 groups: control group1 – intact rats (C); 2–7 – groups of animals with experimental diabetes (ED): 2 – animals with ED withdrawn from the experiment on the 21 day (ED1); 3 – animals with ED withdrawn from the experiment on the 35 day (ED2); 4 – animals with ED + preventive introduction of selenium chrome lipid complex (ED+Pr); 5 – animals with ED + therapeutic introduction of selenium chrome lipid complex (ED+Th); 6 – animals with ED + preventive and therapeutic introduction of selenium chrome lipid complex (ED+Pr+Th1); 7 – animals with ED + introduction of chrome chloride ($CrCl_3 \cdot 6H_2O$) and sodium selenit (Na_2SeO_3) for therapeutic purpose (ED+Th2).

Scientific data taken as a basis [12], we decided to model D2 in 2 stages, that is at the background of obesity in experimental animals. Stage 1 – modelling of alimentary obesity. Experimental model of alimentary obesity for groups 2 – 7 was simulated by 4-week use of food attraction inductor – glutamic acid sodium salt at 0.6:100.0 ratio, and high-calorie diet of standard food (47 %), condensed milk (44 %), corn oil (8%) and vegetable starch (1%) – diet #C 11024, ResearchDietes, NewBrunswick, NJ [13]. The control group (C) received conventional food and had free access to water throughout the experimental period.

Simulation of alimentary obesity was monitored by weighing, measuring nasal-anal length and calculating body weight index (BWI) – dividing body weight (in grams) by body length (in centimetres) squared [14].

Used as a basis for the choice of experimental model of streptozotocin-induced diabetes were the recommendations by A.A. Spasov et al. [15], who found increased resistance of Langerhans islets' β-cells to the damaging effect of streptozotocin, provided nicotinamide had been previously introduced. This makes possible to simulate the condition, maximally related to type 2 diabetes which reveals itself in moderate and stable hypoglycemia as well as in the presence of urine in glucose without signs of acidosis.

According to research data [16, 17], biochemical findings enable to interpret developing diabetes on the 21 day since cytotoxin has been introduced.

The next stage of diabetes modelling was single intraperitoneal introduction of «Sigma» (USA) streptozotocin after 24-hour starvation and free access to water on the basis of 65 mg/kg (on 0.1 citrate buffer, pH=4.5), preceded 15 minutes by intraperitoneal nicotinamide (230 mg/kg in physiological solution). Control rats were introduced citrate buffer only. Starting from the very first day of streptozotocin and nicotinamide introduction, group 4 rats were subjected to 21-day daily intragastric introduction of 1 ml 1% water starch solution, containing chlorella-derived lipid complex of selenium (0.6 mcg), chrome (1.05 mcg) and lipids (0.5 mg), that is correlative with everyday physiological standards of the elements' intake [18, 19]. Group 5 rats were being introduced suspension similarly for 35 days. Group 6 animals were being introduced selenium chrome lipid complex for 14 days, starting on the 21 day since cytotoxin ntroduction. Within 21-35 days, group 7 rats were being introduced starch solution of sodium selenit and chrome chloride, containing the similar daily dose of

these microelements in terms of Se^{4+} and Cr^{3+}. For experimental purity, groups 1 and 2 were being injected *pero*physiologic solution for 21 days, whereas group 3 – for 35 days. Groups 1, 2 and 4 were euthanized under thiopental anaesthetic on the 21 day, and groups 3,5,6 and 7 – on the 35 day.

Blood serum and liver were taken for the research. Blood samples were taken from the heart and centrifuged for 30 minutes at 3000 rounds/min. The blood serum obtained (supernatant) was used for the research. Separated liver (500 mg) was used for obtaining the homogenate by the method of differentiated homogenizing, preceded by perfusion with 5.0 ml of physiologic solution. Diabetes development was confirmed by the determination of blood and urine glucose content, urine ketone bodies, tolerance degree to glucose load. In addition, the degree of non-enzymatic protein glycosylation was assessed by fructosamine level in the blood serum [20].

Blood glucose content (mmol/l) was determined with a glucometer "Accu-Chek Active" ("RocheDiagnosticsGmbH", Germany), whereas urine glucose content ("Glucotest", %) and ketone bodies content ("Acetone test, mmol/l") were assessed with "PVP "Norma" test-strip.

Glucose tolerance test was performed in the morning on the 14 day of diabetes development. Blood samples were taken from the caudal vein. Prior to and after glucose load, blood sampling and assessment of glucose content were performed (fasting – zero point, 30, 60, 90, 120 minutes after glucose intake). Glycemic curve was plotted on the basis of test findings.

Lipid profile test (cholesterol, high density and low density lipoproteins concentrations) was performed by enzymatic methods with an assay kit "FelicitDiagnostic" (Ukraine).

Degree of endogenous intoxication was assessed by medium molecules content (MMC) in the blood serum [21] through the isolation of acid-soluble fraction of medium molecules, followed by the detection of tenfold solved supernatant fluid on the SF-46 spectrophotometer, at wave lengths 254 and 280 nm. The research findings were processed with a programme Statistica 6.0 using the methods of variation statistics.

Research findings. Chromatographic and mass-spectrometric analysis of selenium-containing lipids *Chlorella vulgaris* [22], grown at high Se(IV) concentrations, revealed the presence of selenium in all lipid fractions, the mechanism of selenium inclusion remaining unclear. Yet, included selenium and metals are tightly bound to lipids, since a number of these microelements is found following isolation procedure. Most likely, this bond is both the result of microelements' adsorption and their inclusion into lipid molecules at the site of double bond in unsaturated fatty acid or due to intermolecular co-ordination [3], thus making possible to regard these complexes as chemically homogenous and phisiologically adequate.

Previous findings [2] indicate that feeding mongrel albino male rats (b.w. 160 – 180 g) once a day for 14 days with starch solution of Se-Cr-lipid complex (1 ml containing 1.85 mcg selenium, 1.1 mcg chrome and 0.5 mg lipids) would not c result in intoxication, since the content of medium molecular peptides 1 (MMP1) was found to decrease 1.6 times whereas that of MMP2 – 1.4 times. Besides, both liver and blood serum revealed decreased malonic dialdehyde and diene conjugate content alongside increased energy status (increased succinate dehydrogenase and cytochrome oxidase activity). In addition, glutamate dehydrogenase way of glutamate formation was found to become more intense, along with increased catalase activity and reduced glutathione content.

Determination of blood glucose content for the assessment of diabetes development in rats (Fig.1)

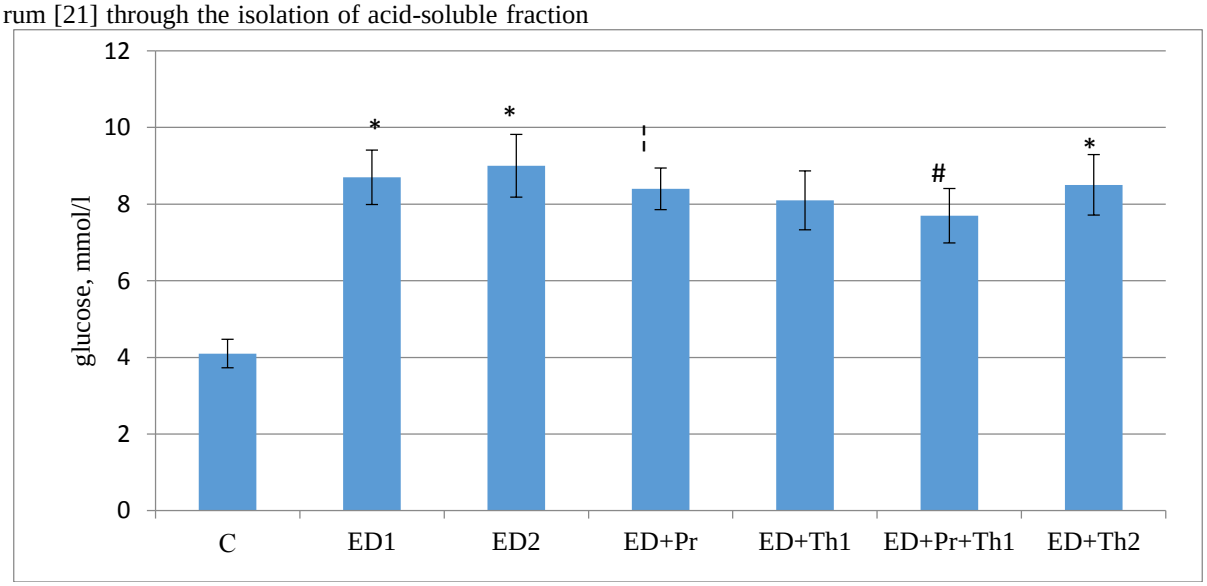


*Fig.1. Effect of selenium chrome lipid complex on the glucose concentration in the blood of rats (M±m, n=12). Comment: here and in figures 3–5 index difference is reliable (p<0.05 by Student's t-criterion) relative to: * – control group (C), ¦ –D1 group; # – D2 group.*

For the intact rats, the index was 4.1±0.1 mmol/l, whereas for the ED animals it increased 3.8 times (15.9±0.33 mmol/l) on the 3d day, 11.5±0.29 mmol/l – on the 7th day and 8.9±0.23 mmol/l – on the 14th day.

From this point on, glycemia level remained almost invariable – 8.7±0.21 mmol/l and 9.0±0.23 mmol/l on the 21st and 35th day, respectively.

Assessment of urine glucose content revealed similar dynamics. For intact rats, the index was 0%, the growing to 0.5% on the 3-7 days after cytotoxin introduction, and later on – 0.1%. However, research of urine ketone bodies content gave a negative result, indicating the lack of ketoacidosis that is typical for type 1 diabetes.

According to WHO data [23], diabetes is diagnosed at fasting glucose concentration in venous or capillary blood > 6.1 mmol/l. Two hours after glucose tolerance test (GTT) or at random determination, diabetic glucose concentration in the capillary blood > 11.1 mmol/l, and in venous blood >10.0 mmol/l. Ensuing from GTT curves' analysis is that animals with ED2 revealed 13 mmol/l higher glycemia level 2 hours since glucose introduction (Fig.2).

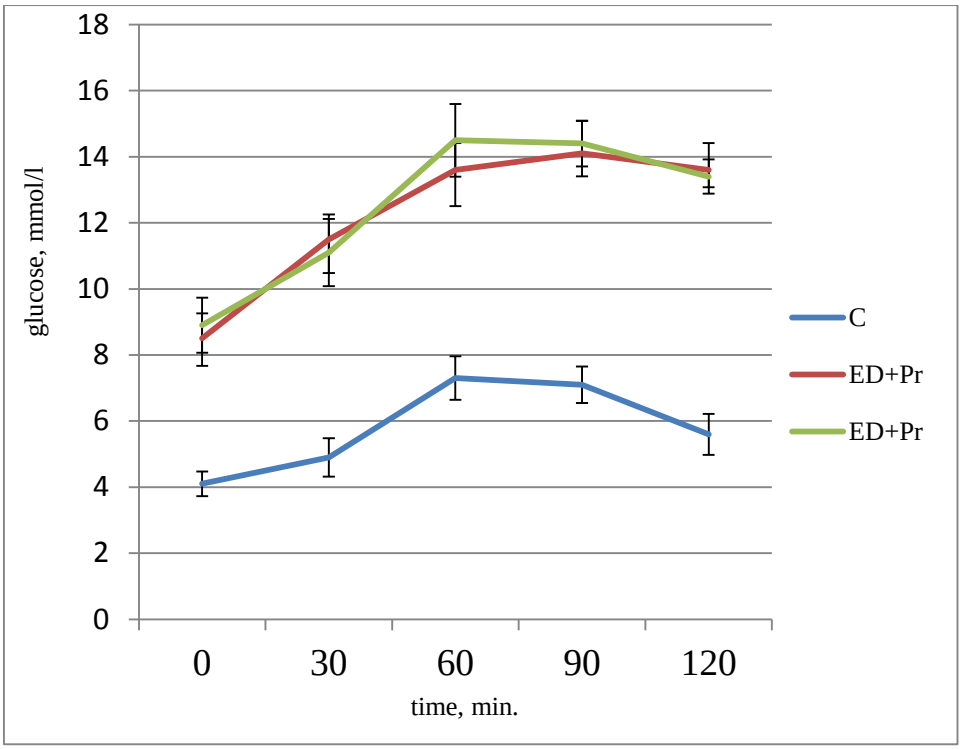


Fig.2.Glycemia dynamics at oral GTT in rats (M±m, n=12).

Maximum increase of glucose blood content in C and D groups, nearly twice as the concentration index for the control group, was found 60 min after glucose load. The index barely changed within the next 30 min, and was found to be gradually decreasing thereafter. Research findings confirm decreased tolerance to glucose in this group, indicating disturbed glucose transportation to the cells and developing insulin resistance.

The degree of glucose homeostasis disturbance is additionally confirmed by an Amadori product – fructosamine, widely used for the evaluation of non-enzymatic protein glycosylation. It is more dynamic than glycated hemoglobin, that characterises long-term (2 – 3 months) control of diabetes. Fructosamine concentration, with two-week half-life, is capable of representing early glucose homeostasis disturbance. Therefore, its use is grounded by the specifics of this research. As can be seen from Fig.4, diabetic rats revealed significant increase (1.88 times) of blood serum fructosamine concentration (ED1 group) that is indicative of activated non-enzymatic glycosylation and intensified glucose metabolization through hexosamine (fructose formation) in insulin- resistant tissues [24].

In diabetes, disturbed carbohydrate metabolism is concomitant with marked lipid metabolism changes – increased low density lipoproteins' (LDLP) content (1.3 times) and cholesterol (2.0 times). Besides, persistent intoxication is observed (1.7 and 2.2 times increase of medium mass 1 (MM1) and medium mass 2 (MM2) content, respectively).

Thus, research findings prove that our procedure can be used as an adequate type 2 diabetes model.

Introduction of selenium chrome lipid complex resulted in reduced systemic intoxication (Fig.3): group ED1 revealed decreased MM1 (by 15.7%) and increased MM2 content (by 23.5%); as compared with ED2 group, MM1 content in groups ED+Th1, ED+Th2, ED+Pr+Th decreased by 9.9%, 5.6% and 22.5%, respectively, whereas MM2 content increased by 17.9%, 9% and 37.4%, respectively.

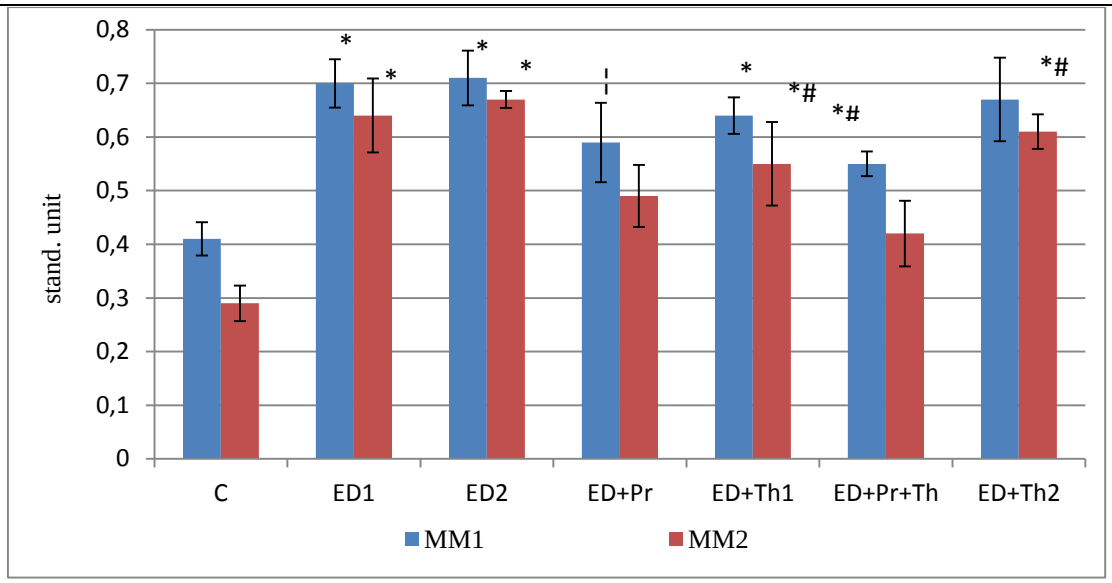


Fig. 3.Rat blood serum MM content б) (M±m; n =8–13).

Introduction of selenium chrome lipid complex to rats improves carbohydrate metabolism. According to the data in Fig.1 and Fig.4, group ED+Pr rats revealed decreased blood glucose content by 3.5% and fructosamine – by 9.6%, as compared with ED group. In comparison with ED2 group, glycemia degree for groups ED+Th1, ED+Th2 and ED+Pr+Th was found to decrease by 10%, 5.6% and 14.5%, respectively, whereas fructosamine level decreased by 6.7%, 5.5% and 12.2%, respectively.

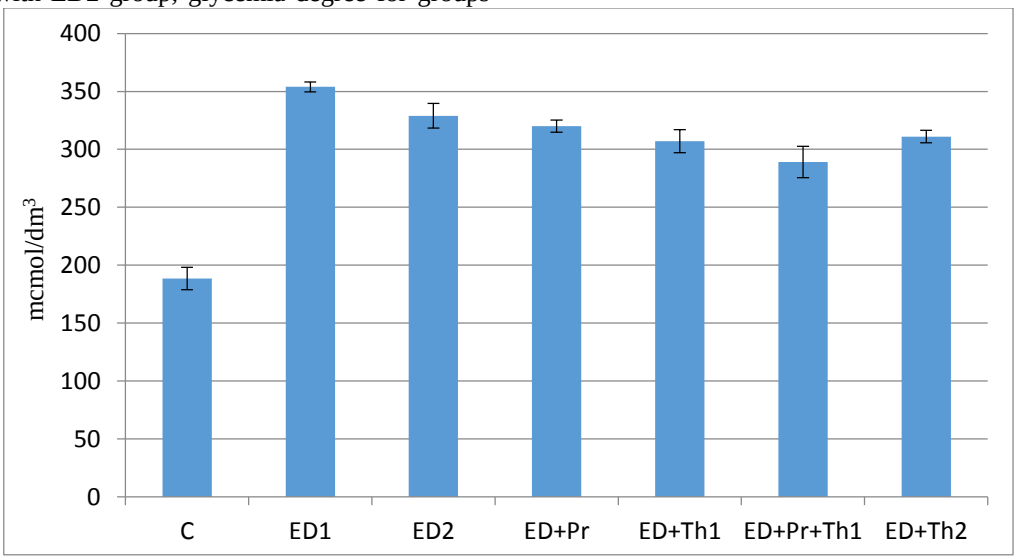
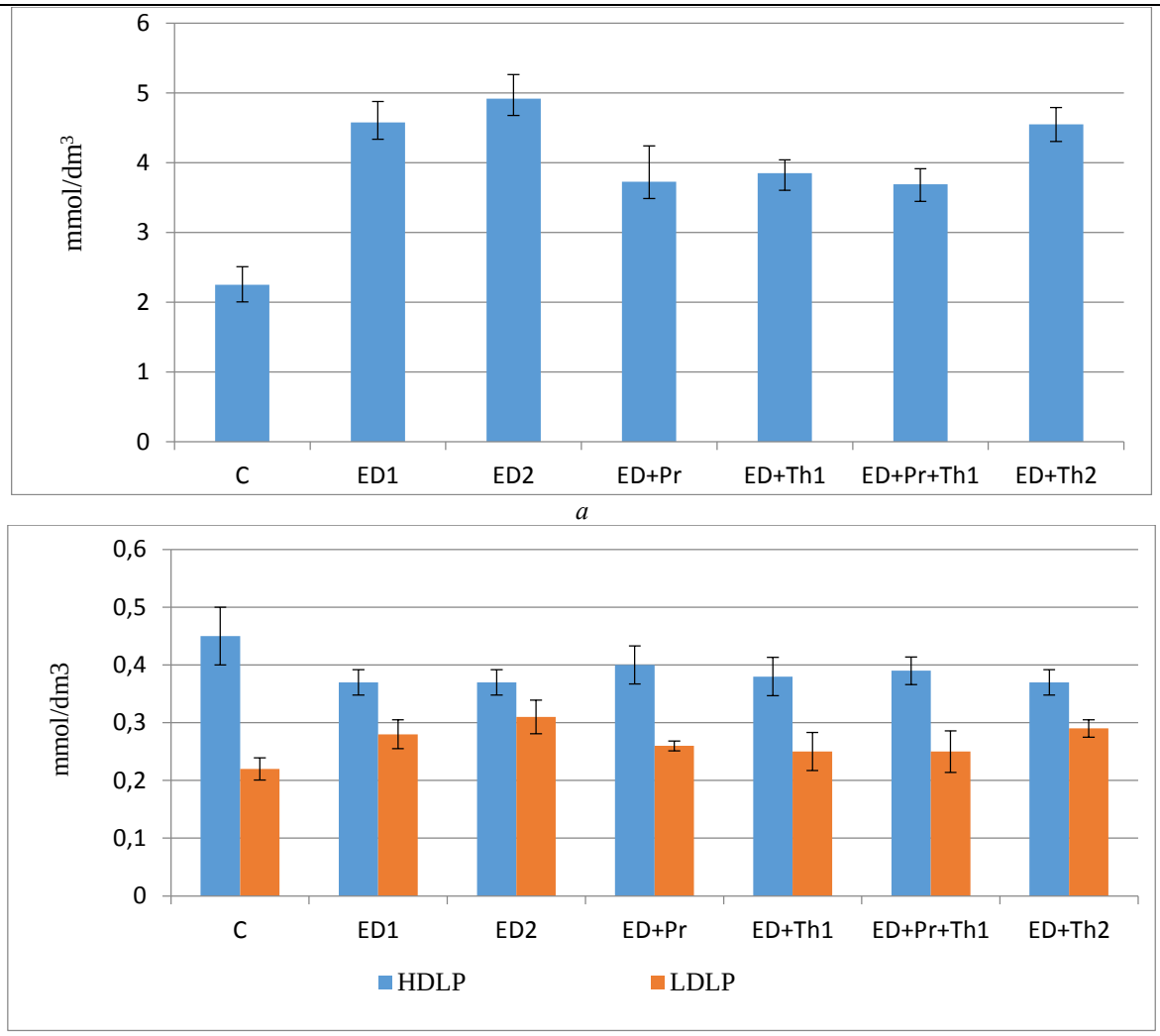


Fig. 4 Fructosamine concentration in rat blood serum (M±m, n=8–9).

Improved lipid metabolism is noted (Fig.5): as compared with ED1 group, ED+Pr group revealed decreased cholesterol and LDLP content by 18.6% and 7.1%, respectively; by 21.8% and 19.4 % – in ED+Th1 group; by 7.5% and 6.5 % – in ED+Th2 group; by 19.5% and 19.4% - in group ED+Pr+Th1. High density lipoproteins' content (HDLP) was found to have increased: in ED+Pr group – by 8.1% as compared with D1 group; in comparison with ED2 group, the values in ED+Th1 and ED+Pr+Th1 – by 2.7% and 5.4%, respectively. HDLP indices in ED+Th2 group remained stable.



а

б

Fig.5 Systemic cholesterol content (a) and cholesterol in rat blood serum lipoproteins (b) (M±m; n=8–9).

As seen before, chorella posesses antioxidant activity and may be helpful in preventing diabetes and diabetic complications [25], along with producing hypoglycemic effect[26].

We have previously established the regulatory role of the substance under research in intact rats, regarding energy exchange and the state of oxidative processes [27,28]. Normalizing properties of chlorella-derived selenium-metal drugs may be realised through their inclusion into lipids [29] and "screening" of their peroxidation, when biological effect of selenium accumulation reveals itself in providing non-enzymatic way of lipids' antioxidant protection at the background of decreasing role of catalase and superoxide dismutase in the antioxidant protection [30].

The research findings are indicative of selenium chrome lipid complex decreasing intoxication status and effecting essentially metabolism in rats under experimental diabetes.

Summary. Uncovered basic index changes of carbohydrate and lipid metabolism are in line with the clinical presentation of developing diabetes and are concordant with metabolic disturbances in insulin-independent diabetics. Therapeutic and preventive introduction of chlorella-derived selenium chrome lipid complex in progressing streptozotocin diabetes contributes to the normalization of a series of metabolism indices and to the reduction of concomitant intoxication background. Basing on research findings, it is acceptably to assert that the implication of chlorella-derived selenium chrome lipid complex provides considerably higher therapeutic effect in diabetis, being more effective as compared with non-organic compounds, which are much less assimilated in the body. The findings obtained are promising in view of the implication of biologically active chlorella supplements with Cr^{3+} and Se^{4+} ions for the correction of diabetic pathology.

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