

Biological effect of hydroxycitric acid within a *Garcinia Cambogia* extract on the nutrient metabolism

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Abstract. Among plant supplements, which once included in a food regimen, induce a favorable evolution in persons seeking to lose some weight, *Garcinia cambogia* fruit containing hydroxycitric acid deserves close attention. The aim of this study consisted in the investigation of a biological effect of hydroxycitric acid within a *Garcinia cambogia* extract on the nutrient metabolism. The study involved the use of a *Garcinia cambogia* extract comprising hydroxycitric acid in the amount of 60.23%. In view of simulating the conditions of digestion, model dietary media consisting of extrusion products added with a *Garcinia cambogia* extract and enzymes were developed. The simulation of digestion processes in experimental model media has shown the decrease of glucose formation. Possibly hydroxycitric acid inactivates the activity of amylases, what results in the decline in the content of free monosaccharides and in the reduction of synthesis of glycerol as a fat component with the effect of an inhibited triglyceride formation. The addition of an extrusion product comprising the *Garcinia cambogia* extract to the diet of laboratory animals (rats) after 28 days of the experiment resulted in a reliable reduction of blood total cholesterol and triglycerides by 13% and 28%, respectively, and also in the decrease of body weight of animals by 5.8%. The investigation confirms the data available in the literature on the properties of hydroxycitric acid within a *Garcinia cambogia* extract, which influences the nutrient metabolism, thereby allows using brindleberry processing products for the correction of body weight.

Key words: *Garcinia cambogia*, extrusion product, fats, hydroxycitric acid, metabolism, overweight.

INTRODUCTION

In the recent years the nutrition structure of contemporary population has undergone negative changes: the increase of the share of refined food products, the incorporation in the diet of semi-finished products and fast food with a high content of easily digested carbohydrates and trans-isomers of fatty acids. Said factors contribute to the excess of dietary calories over energy expenditure resulting in the growth of overweight and obesity.

According to the global estimation of the World Health Organization in 2016 over 1.9 billion adults over 18 had overweight and over 650 million of these had obesity. From 1975 to 2016 the number of obese people in the world has more than tripled (WHO, 2018).

There is a similar trend in Russia, according to the data of the Federal Research Center of Nutrition and Biotechnology about 60% of women and 50% of men over 30 have overweight, about 26% of Russian people have obesity. Notably that the number of overweight population is continuously increasing: in 2005 the percentage of obese people was 23% and in 2012 was 25.3% (Basharova, 2016).

An overweight and obesity result in the reduction of life expectancy and the decrease of the quality of life, they are the principal risk factors of non-contagious diseases: cardiovascular diseases (heart diseases, stroke, ischemia); diabetes mellitus; musculoskeletal disorders; some cancers (Shutova & Danilova, 2004).

At present there are various methods of weight loss: drug treatment, surgical interference, diet therapy. The most common and preferred method consists in the development of a balanced diet based on the consumption of healthy foods. With the aim of maintaining the overall health, the food industry produces products enriched with vitamin and mineral complexes, dietary fibers, plant extracts and other functional supplements (Sadovoy et al., 2012).

In view of the foregoing, for solving the global problem responsible for the increase of the body weight it is necessary to search for components inhibiting the lipogenesis in an organism and investigate the mechanism of their action. Among plant supplements, which once included in a food regimen, induce a favorable evolution in persons seeking to lose some weight, *Garcinia cambogia* fruit deserves close attention.

Studies of many researchers are related to the investigation of a mechanism of the effect of *Garcinia cambogia* extract on metabolic processes in an organism. Yimam et al. (2019) carried out their study on laboratory animals and determined that a *Garcinia cambogia* extract promoted the suppression of appetite. Downs et al. (2005) has come to a similar conclusion, while determining experimentally that a complex compound of hydroxycitric acid contained in a *Garcinia cambogia* extract with calcium and potassium salts (dietary supplement HCA-SX) enhances the effect of the extract and results in the suppression of appetite and the decrease of the body weight, what is due to the increased availability of serotonin and the declined blood lipid content. Semwal et al. (2015) have determined that substances comprised in a *Garcinia cambogia* extract influence the level of serotonin which is able to enhance gastrointestinal peristalsis and secretory activity what results in a decreased food consumption. The paper of Márquez et al. (2012) presents the results of investigation of the efficiency of a *Garcinia cambogia* extract in the regulation of endogenous lipid biosynthesis. The authors explain the effect by the fact that hydroxycitric acid inhibits the citrate lyase enzyme, which is directly involved

in lipid formation processes. A study was related to the effect of hydroxycitric acid on the decrease of body weight of broiler chicken. Han et al. (2016) have determined that the incorporation of hydroxycitric acid in the amount of 3,000 mg kg⁻¹ in a diet of broiler chicken inhibited the expression of SREBP-1C protein binding a fatty acid synthase. The study of Raina et al. (2016) has determined that in addition to the effect of reducing the body weight a *Garcinia cambogia* extract has anti-inflammatory, antiulcerogenic, antioxidant and hepatoprotection effects. Moreover the papers (Koshy et al., 2001; Hayamizu et al., 2003a and 2003b; Tharachand et al., 2015) also confirm biological properties of a *Garcinia cambogia* extract promoting the decline of the body weight.

Consequently there is no consensus in the literature on a mechanism of action of biologically active substances contained in a *Garcinia cambogia* extract on the nutrient metabolism of an organism resulting in the decrease of body weight.

The aim of this study consists in the investigation of a biological effect of hydroxycitric acid (HCA) within a *Garcinia cambogia* extract (GCE) on the nutrient metabolism.

MATERIALS AND METHODS

The research involved the use of a GCE (manufactured by Eusa Colors Sas, France). Physical and chemical quality parameters are provided in the batch certificate (GCE - 121017): color – white; taste and odor – specific for the extract; weight percentage of hydroxycitric acid – 60.23%; moisture content – 3.63%; ash content – 3.78%. The GCE was incorporated into extrusion products to achieve its uniform distribution in ready products and dietary media. For a uniform distribution of the GCE in dietary media it was introduced into a maize grit extrudate of Beshtau maize hybrid produced in the All-Russia Research Institute of Maize. The use of Beshtau maize hybrid grit is due to a predominant content in the grain of a high amylase starch acting as a neutral carrier for the GCE components. The extrusion treatment was performed with the use of a single screw laboratory extruder under the following conditions: temperature 140–145° C, moisture of raw material 16–18%, screw speed 160 rpm, screw length 40 cm, screw diameter 38 mm, die diameter 5 mm, pressure in the pre-die chamber of the extruder 5.5 to 6.2 MPa. The study was designed to use an extrusion product containing the GCE in the amount of 7% relative to the grit weight (Malkina & Baluyan, 2016).

The content of monosaccharides in dietary media was determined by the ion chromatography with the Thermo Scientific Dionex ICS 5000+ (column CarboPac PA20 3*150, protective column Amino Trap 4*50). A 1 g sample of the extrusion product was added with 10 mL of deionized water, carefully mixed until the complete dissolution, added with 5 mL of trichloroacetic acid to precipitate protein substances, centrifugated for 20 min and subsequently filtered. The obtained filtrate was diluted tenfold and the samples were used for the analysis.

The fatty acid composition of dietary media was determined by the gas-liquid chromatography with the use of the instrument HP 4890 with a flame ionization detector. The operating parameters of the instrument: evaporator temperature: 240° C; detector temperature: 270° C, fused silica capillary column HP-INNOWAX, 30 m×0.25 mm, film thickness: 0.25 µm, carrier gas: nitrogen, carrier gas flow rate: 1.5 cm³ min⁻¹. Programming mode of the temperature of the column thermostat is from 50° C to 240° C at the rate of 10° C per minute.

The content of hydroxycitric acid in the extrusion products was determined by the high performance liquid chromatography with 'Agilent 1100' liquid chromatograph provided with a 'VWR' detector. Organic acids were separated in a chromatographic column filled with octadecyl silica gel. The calibration curve was built in coordinates S (peak response) – C (acid concentration), g dm⁻³ within the concentration range of 0.1 to 40 g dm⁻³. The concentration of hydroxycitric acid was calculated from chromatographic peak responses obtained with the use of a spectrophotometric detector at $\lambda = 210$ nm (Wavelength = 210 nm) according to the external standard method.

The biological effect of the GCE on nutrient metabolism was evaluated on laboratory animals in conditions of a fundamental research laboratory for experimental immunology, immunopathology and immunobiotechnology of the North-Caucasian Federal University. The experiment was performed on Wistar male rats of 280–340 g. Experimental tests with laboratory rats were carried out in accordance with the generally accepted ethical standards for animal experimentation in compliance with the rules adopted by the European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes (1986).

During the experiment the animals were kept in standard conditions of a laboratory vivarium in similar care, nutrition, light and temperature conditions with a free access to water and feed. With the purpose of adaptation, the animals were kept in cages in groups for 14 days prior to the study and received equicaloric diet. During this period their physical condition was evaluated every day by a visual examination.

The experiment included the preparation of pills containing the extrusion product. To this end the grinded extrusion product comprising the GCE was mixed with the same amount of flour in all cases. The pills were prepared every day directly prior to feeding the animals. The prepared pills were given once a day 2 hours before the basic diet was placed in the cages. Pill eating by the animals was monitored. Blood samples were collected from the tail vein of the rats with a subsequent serum separation in a bench-top centrifuge MicroCL 17R (Thermo) at 3,000 rpm for 15 min. Total cholesterol and triglycerides in the blood serum were determined with a biochemical semi-automated analyzer BioChem SA Plus (HTI) with the use of reagent kits manufactured by High Technology (USA). The obtained results were statistically processed using the Student's test. The samples were analyzed in triplicate. The calculations were performed with the use of Biostat software (version 4.03).

RESULTS AND DISCUSSION

The preparation of the extrusion product included a coextrusion of Beshtau maize hybrid grit and the GCE in an amount of 7% of the grit weight. During the extrusion processing a plant raw material is exposed to physical factors such as a high temperature, pressure, mechanical stress. Physical factors may result in the neutralization of biological activity of the GCE component. In view of this, the preservation rate of hydroxycitric acid in grain extrusion products was determined by the HPLC with the use of a liquid chromatograph 'Agilent 1100' provided with a VWR detector.

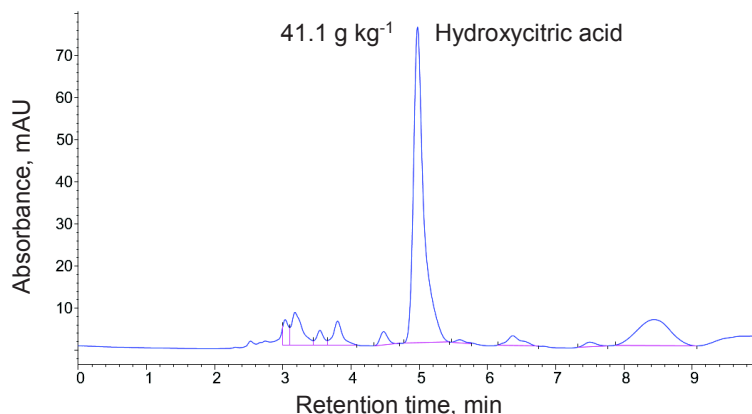


Figure 1. HCA content in the extrusion product containing the GCE.

The extrusion product added with 7% of the GCE was shown to contain hydroxycitric acid in the amount of 41.1 g kg^{-1} . The HCA preservation rate (90%) determined experimentally following the coextrusion with the high-amylose maize grain is due to the HCA insulation from external influences by its incorporation into the matrix structure of gelatinized starch.

For purposes of investigation of possible nutrient metabolism variants, model dietary media were prepared with the use of the extrusion product and enzymes (Table 1).

Samples of the extrusion products were grinded, added with an amount of water necessary to achieve the moisture content of 50% and placed to an incubator at the temperature of 37°C . In samples 1 and 5 the pH was 6.8–7.0 with the incubation time of 60 minutes, for the amylase activity is maximal in a slightly alkaline medium. For ensuring the optimal effect of trypsin (samples 3 and 7) a slightly alkaline medium was also generated with the pH of 7.8–8.0 and the time of incubation was 120 minutes. Samples 2 and 6 were incubated for 180 minutes at pH of 7.6–7.8 to provide an active effect of lipase on a respective substrate. For samples 4 and 8, where the exposure to all three enzymes has to be ensured, optimal conditions for each enzyme were created gradually. Once the model dietary media were incubated, the amount of monosaccharides and a fatty acid composition of the media were determined.

Fig. 2 shows amounts of monosaccharides (fructose, arabinose, galactose, glucose) detected in the model dietary media. The data evidence that with the addition of amylase to a dietary medium polysaccharides are subjected to an enzymatic hydrolysis with the

Table 1. Variants of model dietary media

Sample number	Model dietary media added with enzymes, 0.002% relative to the weight of the extrusion product			
	amylase	lipase	trypsin	amylase + lipase + trypsin
Extrusion product (control)				
1	+			
2		+		
3			+	
4				+
Extrusion product added with the GCE, 7% (experiment)				
5	+			
6		+		
7			+	
8				+

production of glucose. In the experiment with the addition of amylase to the extrusion product containing the GCE (sample 5) the amount of resulting glucose was decreased by 30% in comparison with the extrusion product also containing the amylolytic enzyme but without addition of the GCE (sample 1). When the enzyme complex was used with the extrusion product added with the GCE (sample 8) the decline of the production of glucose by 8% was also recorded in comparison with sample 4. In our opinion, this is explained by the ability of hydroxycitric acid to create conditions for inactivation of amylolytic enzymes, what results in the decline of concentration of monosaccharides involved in fat synthesis (Marshalkin, 2016). The monosaccharides – glucose, fructose, galactose – formed in the hydrolysis of complex carbohydrates can be interconverted under the effect of specific enzymes (Filippovich, 1998).

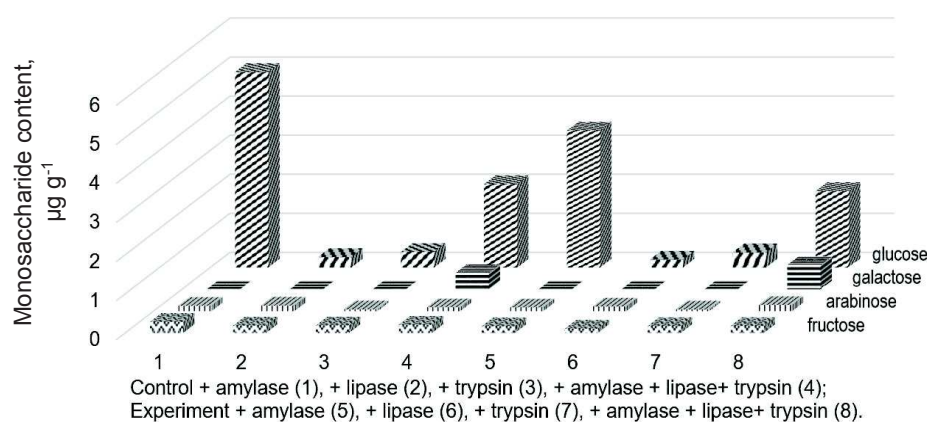
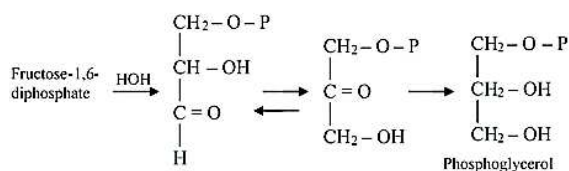


Figure 2. Monosaccharide content in model dietary media.

As follows from the reactions of fat synthesis (Marshalkin, 2016, Bruhman, 1981), the amount of carbohydrates is a crucial factor in the process of forming fats – triglycerides. Their synthesis requires glycerol and fatty acids. Given that neutral lipids are glycerol esters with higher fatty acids, the biosynthesis of both components: glycerol and higher fatty acids should be considered individually.

Glycerol is formed from monosaccharides via the known pathway (Bruhman, 1981), where fructose is used as a substrate for the production of one of the fat components such as glycerol. Under the effect of the enzyme aldolase fructose-1,6-diphosphate breaks down to phosphoglyceraldehyde and phosphodioxyacetone which is easily enzymatically reduced to phosphoglycerol. The formed phosphorylated glycerol directly participates in fat synthesis.



As provided above (Fig. 2) dietary media containing the GCE had a lower carbohydrate content in relation to the samples containing no HCA, what according to the theory of glycerol formation from carbohydrates would result in a reduced glycerol concentration in dietary media.

The biosynthesis of neutral fats takes place in microsomes in the presence of glycerol in the form of glycerophosphate and activated fatty acids. The interaction of active fatty acids and glycerophosphate proceeds stepwise. First two fatty acid residues are attached to glycerol to form phosphatidic acid. Then phosphatidic acid loses the phosphate residue under the effect of the phosphatase enzyme is converted into a diglyceride (Marshalkin, 2016; Bruhman, 1981). Finally, the third active fatty acid molecule is attached to the diglyceride to form a triglyceride.

The neutral fat so synthesized is used in the organism for various purposes, while its excessive portion is deposited in fat depots (Marshalkin, 2016).

Consequently, the provided scheme of fat synthesis from carbohydrates demonstrates that in the case the content of monosaccharides in dietary media is reduced, glycerol synthesis is inhibited, what in its turn results in the decrease of fat concentration.

A fatty acid composition in model dietary media was investigated by a gas-liquid chromatography, the results of the study are presented in Table 2.

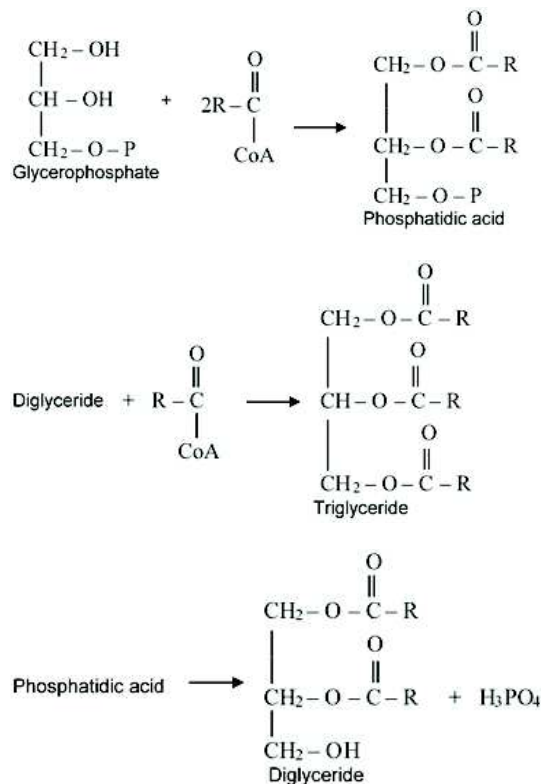


Table 2. Fatty acid composition of model dietary media, % to the sum of fatty acids

Fatty acid	Designation	Extrusion product added with the GCE, 7%			
		Without enzymes	Added with enzymes, 0.002% of the weight of the extrusion product		
			Amylase	Lipase	Trypsin
Myristic	14:00	1.9	1	0.9	1.4
Palmitic	16:00	25	16.8	18.8	16.6
Palmitoleic	16:01	2.1	3.7	1.9	1.9
Stearic	18:00	30.6	8.4	13.9	3.7
Oleic	18:01	10.5	25.5	30.1	30.5
Linoleic	18:02	11.2	31.1	30.7	42.3
Linolenic	18:03	0.6	0.5	0.8	0.5
Arachidonic	20:00	0.5	1.4	1.2	1.7
Content of PUFAs		24.4	60.8	63.5	75.2
Content of UFA		58	27.6	34.8	23.4
Total fatty acids		82.4	88.4	98.3	98.6
Fat, %		0.86	0.82	0.79	0.82

One can see from the data presented in Table 2 that with a combined addition of the GCE and enzymes to a dietary medium the content of polyunsaturated fatty acids is increased. This is a direct evidence of an enhanced degradation of deposited fat under the effect of HCA having an additional hydrophilic oxy-group what contributes to a better solubility of the substrate and hence to the increased velocity of fat hydrolysis reactions.

For confirming the biological effect of HCA within the GCE on the nutrient metabolism the study analyzed the influence of the extrusion product containing 7% of the GCE on blood biochemical indices and changes of the body weight of laboratory animals (male rats) (Malkina et al., 2016).

The animals were divided into 4 groups, six rats in each:

Group I – animals receiving a standard diet (intact control);

Group II – animals receiving a standard diet + an extrusion product containing 7% of the GCE;

Group III – animals receiving a standard diet + an extrusion product without addition of the GCE;

Group IV – animals receiving a standard diet + the GCE.

The animals were fed with prepared pills containing the extrusion product and wheat flour. When an amount of the extrusion product in the pills was selected, the content of the GCE was taken into account. According to the available data, 1,000 mg of the GCE per day was taken as a human therapeutic dose. The human dose was converted into an animal dose in accordance with the manual for experimental (preclinical) study of new pharmacological substances with the use of a conversion factor. Intact control animals (Group I) were only added a flour pill base to the diet. Animals of Group II received once 1,137 mg kg⁻¹ of the extrusion product added with the GCE. Animals of Group III received 1,137 mg kg⁻¹ of the extrusion product, which contained no GCE. Animals of Group IV received 79.6 mg kg⁻¹ of the *Garcinia cambogia* extract (Khabriev, 2005).

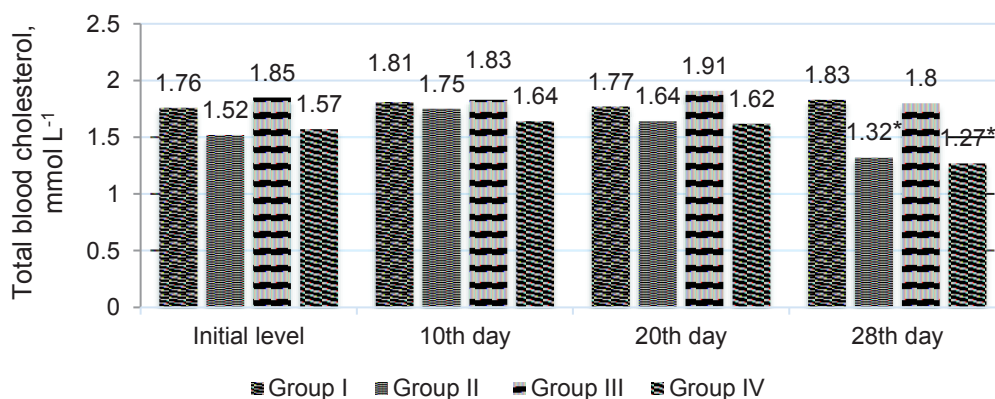


Figure 3. Dynamics of total blood cholesterol.

* – statistically significant changes compared to the initial level at $P < 0.05$.

The data of the experiments showed (Fig. 3) that in comparison with the group of intact control animals (Group I), where no statistically significant fluctuations of lipid spectrum indices was recorded during the experiment, there was a number of changes in the experimental groups.

At the 28th day of the experiment animals of Group IV and Group II demonstrated a reliable decline of total cholesterol respectively by 15% ($P < 0.05$) and 13% ($P < 0.05$) compared to the initial level.

The detected decrease of triglycerides in the blood of rats of Group IV on the 20th and the 28th day of the experiment amounted to 27% ($P < 0.001$) and 23% ($P < 0.05$), respectively. In the blood of rats of Group II the decrease of triglycerides was recorded on the 28th day of the experiment (28%, $P < 0.05$) (Fig. 4).

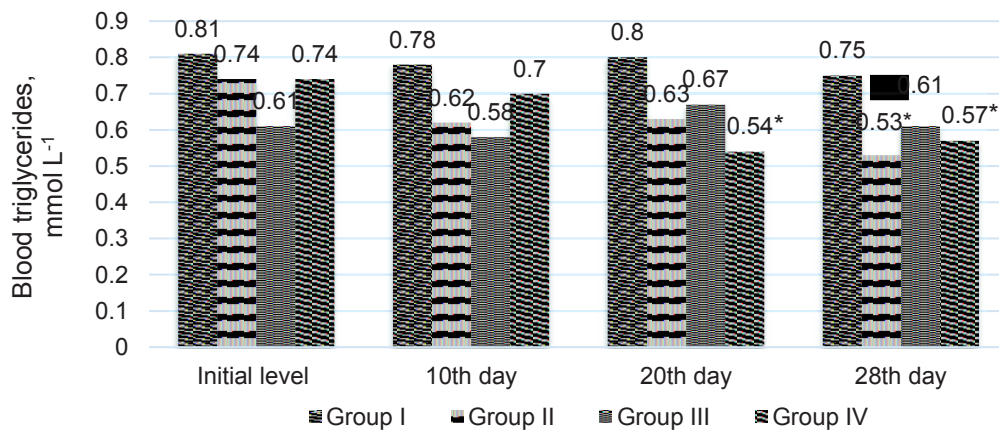


Figure 4. Dynamics of blood triglycerides.

* – statistically significant changes compared to the initial level at $P < 0.05$.

The average blood indices of total cholesterol and triglycerides in animals of Group III and Group I showed no statistically significant changes during the whole experiment.

During the experimental period animals of Group I and Group III showed no significant changes in body weight mass, the increase was 2.3% and 1.1%, respectively. Considering that there is a modest tendency for increased body weight, we can suppose that it is possibly due to natural growth processes of the animals. Animals of Group II and Group IV were characterized by a tendency for decreased body weight by 5.8% and 5.7% at the 28th day of the experiment, respectively. The experiments proved that the addition of the GCE (containing HCA) to an animal diet both within extrusion products and in native form resulted in the decrease of the body weight, what correlated with the blood triglyceride and cholesterol content.

The obtained results characterize the manifestation of biological action of HCA within the GCE, which is expressed in a reliable decrease of cholesterol and triglyceride levels in the blood of animals after the *in vivo* experiment.

CONCLUSIONS

The performed research allows concluding that the mechanism of the GCE action comes down to the manifestation of at least two biological effects under the influence of hydroxycitric acid (the principal biologically active component of the GCE) on the nutrient metabolism in an organism:

- the inhibition of fat synthesis from carbohydrates determined by the inactivation of amylolytic enzymes;
- the promotion of the degradation of fats including these from depots, what is evidenced by the increased amount of free fatty acids in dietary media containing the GCE and also a reliable decrease of total cholesterol and triglycerides in the blood of animals.

The use of an extrusion product containing the GCE in *in vivo* experiments resulted in a reliable decline of blood total cholesterol and triglycerides and also in a reduction of body weight of animals, what supports the data available in the literature on *Garcinia cambogia* properties facilitating the correction of the body weight.

The performed investigation of the biological action of hydroxycitric acid within the *Garcinia cambogia* extract is a theoretical substantiation of its application in food technologies as a biologically active ingredient for correcting the body weight, what will promote the health of the population.

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