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Lipid Composition of Blood and Liver Cells under Consumption *Trigonella Foenum Graecum L.* on the Background High-Calorie Diet

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Abstract: Comparative evaluation of blood lipids content and lipid composition of cytosol membranes and plasma membrane of hepatocytes were carried out in the normal and under conditions of high calorie diet fed rats. It was shown corrective effect powder seeds *Trigonella foenum graecum L.* on the lipid composition of blood serum of rats with obesity, lowering TG (24.6%), LDL (25%), VLDL (23.8%), cholesterol (16%) and increased HDL levels (25.4%). Our experimental data indicate that feeding the animals high calorie diet supplemented with fine powder fenugreek affect the lipid composition of blood and liver cells, by directing the values of indicators to the levels of the control animals: increase of 70% phosphatidylcholine, 60% phosphatidylinositol and phosphatidylethanolamine, decrease to 56.5% content of sphingomyelin compared to a group of experimental obese rats. Our results allows us to consider *Trigonella foenum graecum L.* as a possible remedy for the prevention of obesity.

Key words: High calorie diet, blood lipids, hepatocytes membranes lipid composition, *Trigonella foenum graecum L.*

1. Introduction

Increasing the frequency of obesity among the population of developed countries, becomes an alarming rate, so this phenomenon can be compared to the epidemic [1]. Obesity is characterized by violations of various body systems, including the regulation of lipid, protein, carbohydrate metabolism. Therefore, the search for new ways of preventing obesity is important and necessary trend of modern medicine and science. Herbal products, which are characterized by a wide range of medicinal properties and low toxicity, in recent years, becoming increasingly used in medical practice, especially for prevention. One of these plants is *Trigonella foenum graecum L.*, which is used for centuries to treat various pathologies in many countries. *Trigonella foenum graecum L.* has different pharmacological activity (has anti-diabetic, antiatherosclerotic, anti-inflammatory, detoxification properties) [2-5].

In recent years, the role of installed large number of external chemical factors in the development of obesity, the consumption of foods that undergo diverse technological processing. Contemporary man loses vital nutrients, trying to compensate for their shortage of food. Replacing quality number is certainly flawed. The excess fats, carbohydrates and calories, deficiency of essential nutrients inevitably leads to obesity. Enriching the diet with vitamins, trace elements, minerals, beneficial fatty acids and other essential nutrients is considered as an effective way to prevent obesity.

Long-term medical practice has proved that the herbal treatment of diseases performance not inferior synthetic analogues, and the absence of side effects and contraindications have advantages. Preparations of natural origin can be eaten in a long time, they stabilize homeostasis, increase the overall non-specific

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resistance of the organism, often detoxification effect, causing activation of antioxidant processes and capable of immunoglobulin and gemostimulation.

Features of metabolism at obesity can be the basis for the study of biologically active compounds of plant origin for the prevention of pathology. The focus of metabolic processes, including chemicals in the cellular and subcellular structures determined by the number and quality of lipophilic food. The composition of fat entering the body largely determine the content of lipid membrane structures of cells that characterizes their functional properties and the ability to maintain structural integrity.

The aim of the study was to recreate at the experiment on rats model of obesity, study the effect of biologically active substances of *Trigonella foenum graecum L.* seeds on some biochemical parameters of lipid content of blood and liver cells under conditions of high calorie diet.

2. Materials and methods

Experiments were carried out in accordance with international bioethical principles of animal experiments, international agreements and national legislation in this field [6]. Animals were kept in a vivarium that was accredited in accordance with the 'standard rules on ordering, equipment and maintenance of experimental biological clinics (vivarium)'. Instruments to be used for research are subject to metrological control.

Animals and housing conditions. Studies conducted on non-linear rats and divided to three groups of 10 animals each. The animals of each experimental group were individually housed in polypropylene cages in an environmentally controlled clean air room, with a temperature of 22 ± 3 ºC, a 12 h light/12 h dark cycle and a relative humidity of 60 ± 5%.

Animal and Diet. The animals of all groups were given water ad libitum. Rats of group 1 (Control, C) were fed by a standard nutrition of vivarium during 14 weeks of the experimental period. The animals of group 2 (HCD) was fed by a high-caloric diet, which contained: standard nutrition (60%), lard (10%), eggs (10%), sucrose (9%), peanut (5%), dry milk (5%), sunflower oil (1%) and water ad libitum [7]. The animals of group 3 (HCD+FNG) was fed by a high-caloric diet, which contained: standard nutrition (60%), lard (10%), eggs (10%), sucrose (9%), peanut (5%), dry milk (5%), sunflower oil (1%) and fenugreek seed powder (2%) during 14 weeks of the experimental period. Food consumption of animals in each group was measured daily at the same time (09:00 to 10:00 h) and body weights were determined once a week. Body mass index (BMI) = body weight (g) / length^2 (cm^2).

Seed powder of *Trigonella foenum graecum L.* varieties Ovari 4 were provided by Professor of the University of West Hungary Sándor Makai (Institut of Crop Sciences, Department of Medicinal and Aromatic Plants). It was determined by photon correlation spectroscopy using a laser correlation spectrometer, that the mean hydrodynamic diameter of particles *Trigonella foenum graecum L.* was 30 nm.

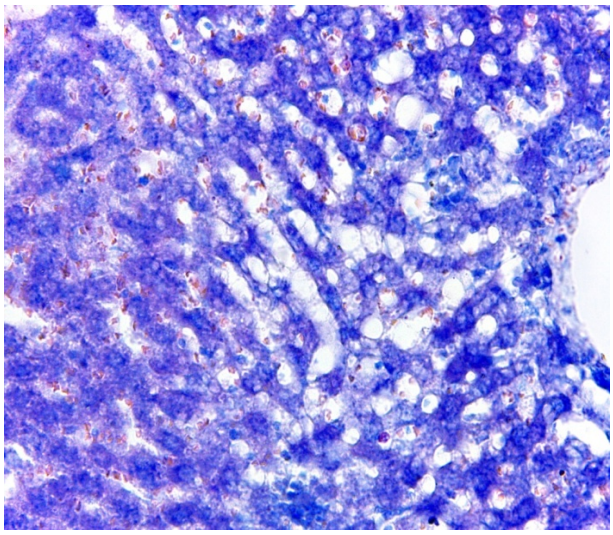
Isolating of the cytosol membranes and plasma membrane (PM) hepatocytes were carried out according [8]. Determination of qualitative and quantitative composition of lipids of plasma membranes was carried out according [9]. Serum samples were tested for cholesterol, low-density lipoproteins, very low density lipoproteins, high-density lipoproteins, triacylglycerids on biochemical analyzer Microlab 300 (Elitch, France) using commercial kits firm "Elitch".

A histological control of intact and experimental samples were made. The statistical analysis of the research results perfomed by conventional methods of variation statistics on the basis of 10 repetitions (M ± m, n = 10). Reliability of performance differences was assessed using Student's t-test, assuming the significance level at < 0.05.

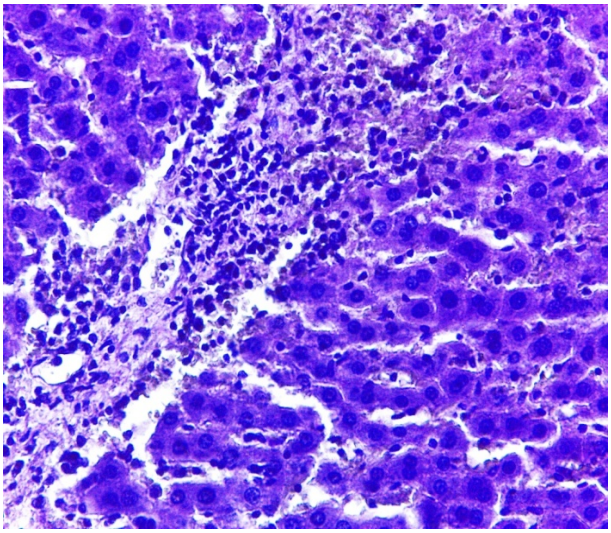
3. Results and Discussion

Since the beginning, we have reproduced the model of obesity in rats, which confirms our findings of histological studies.

Hepatic Histopathology. Fig. 1 represents liver histopathologies of experimental rats. The histopathological examination of HCD group showed signs of granular and vacuolar degeneration (Fig. 1a). Small foci of lymphocytic infiltration were located mainly in the stroma, in the course of the small arteries and veins increases the amount of tissue basophils. It was observed local periportal proliferation stroma. When high-calorie diet was supplemented with 2% of the fineseed powder *Trigonella foenum graecum L.* (FNG) in the liver revealed a weakly pronounced features of granular dystrophy of hepatocytes, lymphocytic infiltration is almost not observed (Fig. 1c). The glycogen level in the cytoplasm of parenchymal cells in comparison with



(a)



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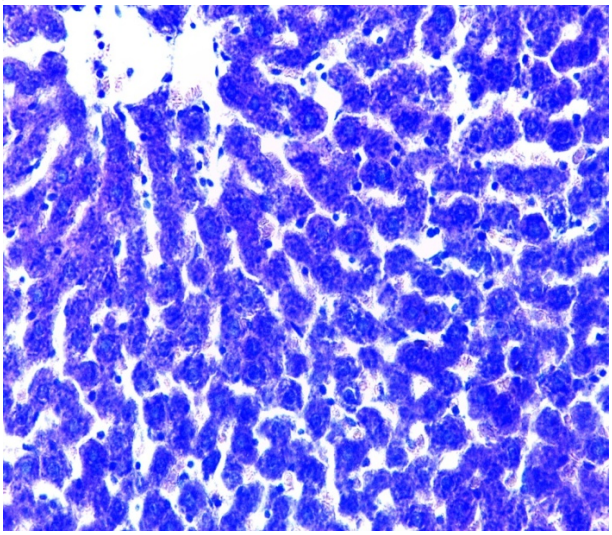

(c)

Fig. 1 Effect of *Trigonella foenum-graecum L.* seed powder on hepatic histopathological changes in high calorie diet- (HCD-) induced obese rats. Group (a): rats were fed HCD (a), showing fatty degeneration and greater hepatic lipid accumulation; Group (b): intact rats were fed standard nutrition of vivarium supplemented with 2% fenugreek seed powder (Control + FNG), showing mild congestion, no fatty changes, and less hepatic lipid accumulation; Group 3 (c): rats were fed HCD supplemented with 2% fenugreek seed powder (HCD + FNG), showing no fatty changes and considerably lower hepatic lipid accumulation.

the above observation increased. At the same time, only some animals receiving a standard diet of vivarium with the addition *Trigonella foenum graecum L.* (Fig. 1b) were observed mildly expressed granulation of the liver cells, sporadic congestion of lymphocytes.

The next stage of our research was to study the lipid composition of the blood and liver cells in diet-induced obesity and effect of the fine seed powder *Trigonella foenum graecum L.*

The results of our research showed that in the blood of rats that were on high calorie diet, cholesterol (C), triacylglycerides (TG) and low-density lipoproteins (LDL), very low density lipoproteins (VLDL) significantly increased respectively by 1.3, 1.9, 1.4 times, buthigh-density lipoproteins (HDL) cholesterol content reduced of 1, 9 times compared with those of control animals (Table 1). Thus, the feeding animals of high calorie diet for 14 weeks caused dyslipidemia in rats. We also found, when administered in high calorie diet fine powder fenugreek seed, in the blood of rats cholesterol, triacylglycerides, LDL, VLDL statistically significantly decreased, and HDL levels increased as compared to animals fed with only a high calorie diet (Table 1).

As other researchers have shown that the use of fenugreek seeds leads to a reduction in the number of biomarkers of oxidative damage, cholesterol and triacylglycerols levels [10-12].

After analyzing published data and our results we can assume that *Trigonella foenum graecum L.* has the ability to modify lipid metabolism and reduce the risk of developing obesity complications.

The liver plays a central role in metabolism of lipids, the major metabolic transformations of which are made in the cytosol membranes and PM hepatocytes, therefore these structures were the main subjects of our further research.

Our studies of lipid content of rat hepatocytes found that in plasma membranes liver cells cholesterol level increased of 1.7 times, triglycerides 1.8 times, lipid composition of cytosol fraction increased respectively 1.8 and 1.9 times in high calorie diet in Fig. 2 and Fig. 3.

Probably due to excess fat in the of high calorie diet lipids can't be transported and stored in adipocytes, and begin to accumulate in the intracellular space hepatocytes - cytosol, which starts the process of fatty liver.

We also found differences of phospholipid composition of plasma membranes and cytosol membranes in hepatocytes of rats fed of high-calorie diet in Figs 2 and 3.

It was found in plasma membranes hepatocytes decreased content of phosphatidylcholine 1.8 times, phosphatidylethanolamine and phosphatidylinositol 1.7 times, but the amount of sphingomyelin 2.5 times and phosphatidylserine 1.8 times increased compared to the control in Fig. 2.

In the cytosol fraction, by contrast, it was decreased content of phosphatidylcholine of 1.6 times, sphingomyelin of 1.4 times, phosphatidylserine of 1.5 times compared to the control. At the same time content

Table 1 Effect of *Trigonella foenum graecum L.* seed powder on serum blood lipid levels in high calorie diet induced obese rats (M ± m; n = 10).

Parameters	Groups		
	C	HCD	HCDFNG
C (mg/dL)	1.50 ± 0.15	1.99 ± 0.15*	1.67 ± 0.09#
TG (mg/dL)	0.62 ± 0.04	1.18 ± 0.09*	0.89 ± 0.06*#
HDL (mg/dL)	0.742 ± 0.065	0.575 ± 0. 041*	0.721 ± 0.038#
LDL (mg/dL)	0.222 ± 0.03	0.308 ± 0.024*	0.236 ± 0.021#
VLDL (mg/dL)	0.124 ± 0.008	0.236 ± 0.018*	0.178 ± 0.01*#

*P < 0.05, statistically significant difference from control;

#P < 0.05, statistically significant difference from high calorie diet induced obese rats.

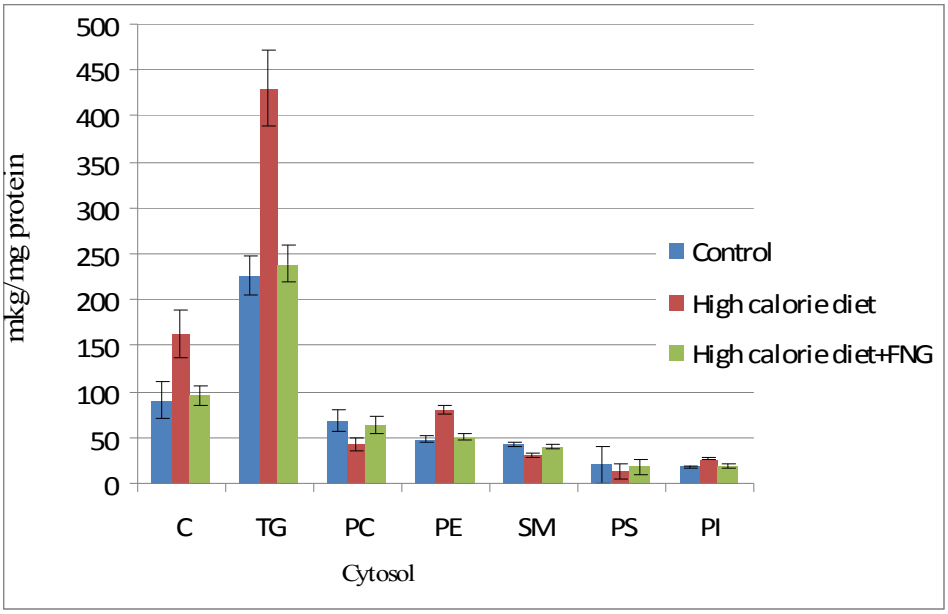

Fig. 2 The content of lipids (μg / mg protein) in fraction plasma membranes of rat hepatocytes of high calorie diet and use *Trigonella foenum graecum L.*

C (cholesterol), TG (triglycerides), PH (phosphatidylcholine), PE (phosphatidylethanolamine), SM (sphingomyelin), PS (phosphatidylserine), PI (phosphatidylinositol).

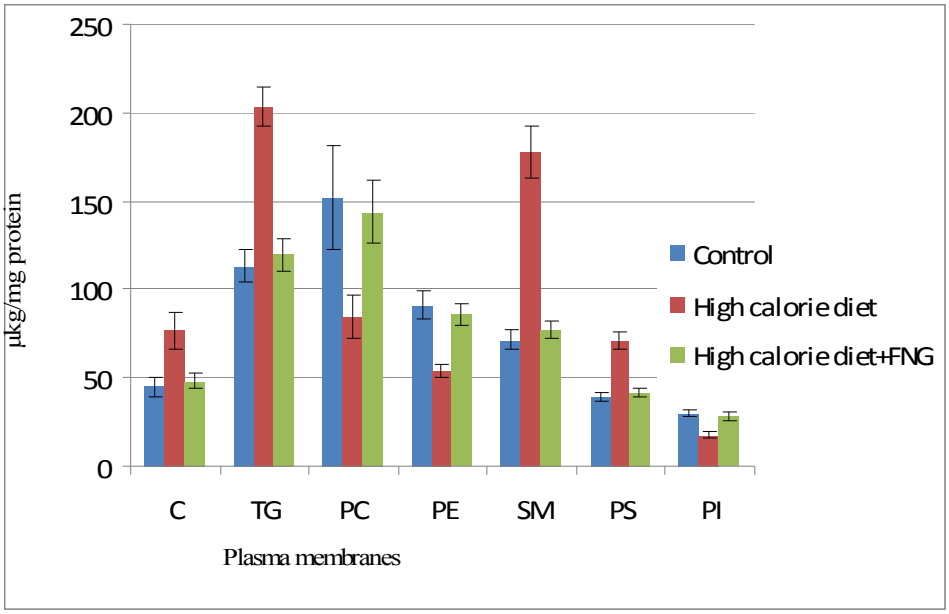

Fig. 3 The content of lipids (μg / mg protein) in fraction cytosol of rat hepatocytes of high calorie diet and use *Trigonella foenum graecum L.*

of phosphatidylethanolamine and phosphatidylinositol in the cytosol fraction increased respectively of 1.7 times and 1.5 times in Fig. 3.

Changes phospholipid content of the studied fractions of hepatocytes may be explained of the activation of lipid peroxidation processes under conditions of high calorie diet.

Perhaps that under prolonged high calorie diet in plasma membranes a hepatocytes homeostatic mechanisms activate biosynthesis of sphingomyelin that support systemic inflammation, oxidative stress.

Long-term consumption of high calorie diet gradually

reduces the detoxication properties of the animals liver contributing to damage cell membranes. This is confirmed by the increasing of phosphatidylserine, sphingomyelin and reduction of phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol in plasma membranes in our studies.

The consumption of high calorie diet supplemented fine powder seeds *Trigonella foenum graecum L.* influence the lipid composition of liver cells: cholesterol content decreased by 1.6 times, triglycerides - by 1.7 times compared to animals fed only the high calorie diet.

Amount phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol increased respectively by 1.7 times, 1.6 times, 1.6 times in plasma membranes hepatocytes. The content of sphingomyelin and phosphatidylserine decreased respectively to 2.3 times and 1.7 times compared with the group animals that fed only high-caloric diet.

Under the same conditions in the cytosol fraction increased number of phosphatidylcholine, sphingomyelin of 1.5 times, phosphatidylserine 1.4 times compared to high calorie diet induced obese rats group of animals. Phosphatidylinositol content decreased 1.4 times, 1.6 times phosphatidylethanolamine. Thus, as evidenced by our data, the content of individual phospholipids was close to that of the control animals. It is known that phospholipids are sensitive molecules that perform adaptive function, that they found most precise and perfect compensatory response to various factors.

It is believed that phospholipids, which enter into an organism consisting the herbal comlex, have a positive effect on lipid metabolism, detoxification function of the liver, restore and maintain cell structure hepatocytes.

The lipid composition of seeds *Trigonella foenum graecum L.* (6-10%) presented phospholipids (phosphatidylcholine, phosphatidylethanolamine) and neutral lipids (tri-, di- and monoacylglycerides), diacylglycerol, acetylated sterylglikoside. *Trigonella foenum graecum L.* seeds contains flavonoids, alkaloids (3%), steroid saponins (5-6%), oleic, linoleic and linolenic acids [4, 13].

Thus, we have found restoring effects on lipid metabolism at consumption of high calorie diet containing *Trigonella foenum graecum L.* Based on our data, we can recommend Fenugreek-supplemented diet to use in order to prevent obesity.

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