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INFLUENCE OF MICRODOSE OF FATTY ACIDS OF *CITRULLUS COLOCYNTHIS* EXTRACT ON LIPOPROTEIDS OF RAT BLOOD WITH DIABETES MELLITUS TYPE 1

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Abstract

Purpose: *To investigate the effect of microdoses of fatty acids (FA) of partially defatted dry Citrullus colocynthis extract on the lipid metabolism of rats with type 1 diabetes mellitus (DM) has been studied.* ***Methods:*** *Raw materials were degreased by extraction in a Soxhlet apparatus under conditions of shortening the evaporation time. With the help of gas-liquid chromatographic research, trace concentrations of FA were revealed in the composition of the obtained dry extract of Citrullus colocynthis.* ***Results:*** *It was found that the introduction of the extract of the Citrullus colocynthis vegetal solution with a dose of 400 mg/kg to rats causes redistribution of the classes of lipoproteins to the values of the control group. The dose of 200 mg/kg – does not cause statistically significant changes in plasma lipoproteins.* ***Conclusions:*** *Finally the Citrullus colocynthis extract containing micro doses of FA in their uniform distribution has a positive effect on lipid metabolism, does not bring to an increase of level of the total cholesterol, does not cause an increase of body weight.*

Keywords: cholesterol; *Citrullus colocynthis*; diabetes mellitus; fatty acids; lipoproteins; triglycerides.

1. Introduction

An urgency of diabetic problems in recent years throughout the world is on the top of the scale of the global epidemic. It is known that today there are 382 million patients with diabetes in the world and according to conservative experts' forecasts in 2030 their number will be 592 million [1]. According to date of the Institute of Endocrinology and Metabolism of the National Academy of Medical Sciences of Ukraine for the ten years, the prevalence of diabetes in Ukraine has grown by one and a half times. The most dangerous consequences of the epidemic of diabetes are its systemic vascular complications, which are the main bring disability and mortality of patients with diabetes [2].

Despite the wide range of pharmacological antidiabetic drugs used for the treatment of diabetes and its complications, now more attention is paid on the using of phytotherapy, which is based on the using of plants as preventive and even therapeutic agents [3]. Plants as sources of phytopreparations have already found application in the therapy of a number of diseases, including diabetes. However, mechanisms which are in the base of their therapeutic effect, as well as the most active active ingredient included in their composition, are unknown [4]. Therefore, the search for new forms of herbal preparations, studies of their influence on the organs and systems of the organism, the particularities of the side effects of medicinal plants are important areas of modern biotechnology in pharmacology, and pharmacy.

2. Statement of problem and analysis of the last researches

Herbal preparations contain a number of active components. Harmonious combination and interaction of biologically active substances (BAS) causes high pharmacotherapeutic efficacy of herbal

preparations. The rich chemical composition allows to influence the various links of the pathological process, ensures the complex action, and helps to reduce the profile of side effects. Due to the receipt of the full complex of BAS in their natural ratio, the optimal choice for peripheral reception in the activity of the functional systems of the body is ensured. In a multicomponent drug, there is a mutual enhancement of the useful pharmacological properties of each ingredient, their compliance with the polyvalence of the pathogenesis of the disease, the effect on the patient's organism in general is corrective [2].

Citrullus colocynthis from the family of *Cucurbitaceae* is a perennial herb, common in African and Arab countries, India and the Mediterranean countries, adapted to the climate of Ukraine. Vegetable raw materials of the *Citrullus colocynthis* of different processing is ucesed as antidiabetic, laxative, insecticidal agent, as an antidote to snake poison, in the therapy of edema, bacterial infection, etc. [5]. Despite the existing data, the mechanisms which take part in the implementation of the action of biologically active constituents of *Citrullus colocynthis* fruits are completely unknown.

In the search of the most effective form of application of *Citrullus colocynthis*, authors propose to use different types of extracts [6]. But there is no consensus onabout which extract is the most effective and safe.

Researchers give very contradictory data about benefits of different types of extracts, their safety and effectiveness. So, *Marwat S.K.* a review of literature sources in which authors tell about the positive impact of various extracts of *Citrullus colocynthis* seeds (water extract, skim water extract, water-methanol extract, ethyl acetate and n-butanol extracts) on the next indicators: glucose tolerance, weight gain, pancreas, liver, kidney, testicles, epididymal fat (adipose testicular fat), diaphragm muscle tissue, serum cholesterol, triglycerides, urea, creatinine, transaminases and alkaline phosphatase in animals with the first type of diabetes - streptozotice-induced diabetes. Some authors conclude that the most pronounced effect in diabetic rats gave the normal aqueous and n-butanol extracts, the lowest is the defatted aqueous extract [6]. *Hii C.S.* has an opposite opinion, and believes that the most of extracts have a positive insulinotropic effect has, except for n-butanol [7].

So, the technology of obtaining of a medicinal product is equally important in the realization of the pharmacological result. Recently, more attention has been paid to the development of extraction forms from vegetable raw materials instead of decoctions and infusions, since it is known that the maximum yield of biologically active substances is provided in extraction preparations, the therapeutic effect due to the accuracy of dosing is increased, the rationality of their use, the prolongation of terms and optimization of conditions storage.

In the experiment, we used the dry fruit extract *Citrullus colocynthis (L.) Shrad.*, Which had been prepared in the laboratory of the Department of Pharmacognosy and Botany of the Bogomolets National Medical University.

The crushed parts of the fruit (shell, pulp, seeds) were mixed with each other for further extraction in the Soxhlet apparatus. Extraction of Soxhlet is recognized as the standard and reference method for achieving complete lipid extraction [8]. This method has many advantages, but its main drawback is a long extraction time – 16-24 hours. The procedure was modified to improve the efficiency and reduce the extraction time by 5-10 times by using a press and raising the temperature. But after aqueous extraction of vegetable raw materials in the obtained dry extract with gas-liquid chromatography, we found trace concentrations of fatty acids (FA). We believe that the reason for the presence in the raw material of a certain amount of lipids after extraction with chloroform in the Soxhlet apparatus may be incomplete solubility of polar lipids - phospho- and glycolipids in organic solvents, which unsaturated and saturated FAs are found in our extract. But there are trace microdoses of FA dry extract *Citrullus colocynthis* that can influence on the lipid metabolism. We found it relevant to investigate the effect of the obtained substance on the lipid metabolism of experimental rats and on the body weight.

3. Purpose of the study

The purpose of the work was to study the effect of microdoses of the FA of partially defatted dry extract of *Citrullus colocynthis* on the lipid metabolism of rats with diabetes.

4. Materials and Methods

The model of type 1 diabetes was reproduced by injecting of streptozotocin to rats intraperitoneally once at a dose of 50 mg/kg. Previously it was dissolved in 0,1

M citrate buffer (pH 4,5). The duration of the experiment is 1 month. Control of the development of hyperglycemia was the level of glucose in the blood – 24,24±0,79 mmol/l. In the control groupthe level of the glucose was 8,03±0,4 mmol/l.

Laboratory animals were in standard accommodations of the vivarium of the Bogomolets National Medical University. The maintenance, care for animals, marking and all manipulations were carried out according to the provisions of the "European Convention for the Protection of Vertebrates used for experimental and scientific purposes" [9]. Conformity of the conducted scientific research with ethical requirements is confirmed by the Commission on Bioethics of the Bogomolets National Medical University according to the order of the Ministry of Health of Ukraine №231 of 01.11.2000.

For the study, 24 white mongrel rats weighing 150-270 g were selected. The "Control" group – healthy rats (N=6) were kept in a vivarium under similar accommodations, as were rats for the experiment. Rats of the "Model" group – with SD have got per os for 14 days water for injections – placebo. Group 1 – rats with diabetes who have got a dry extract of *Citrullus colocynthis* with a dose of 200 mg/kg of mass, a group 2 – rats with diabetes who have got *Citrullus colocynthis* at a dose of 400 mg/kg of weight for 14 days.

The FA composition was studied by gas chromatography on a gas chromatograph "Tsvet-500" (Russia) in the laboratory of experimental research at the Research Institute of ECM of the Bogomolets NMU. The FA peaks were identified by comparison with the time of obtaining the standard FA peaks. Quantitative estimation of FA lipids was carried out by the method of normalizing the planes of the peaks of methyl derivatives of FA and determining their composition in percents.

The study of biochemical indices in rats was carried out in the clinical diagnostic laboratory of the Scientific Research Institute of Experimental and Clinical Medicine (SRI ECM) of the Bogomolets National Medical University according to standard unified methods. The lipid metabolism was studied by biochemical sets for the determination of: total cholesterol – *Cholesterol PAP*, triglycerides – *Triglycerides PAP*, cholesterol-lipoproteins high – *HDL Cholesterol*, and low density – *LDL Cholesterol*, manufactured by *Diagnosicum Zrt (Hungary)* [10].

The statistical processing of the received data samples was carried out with the program *Statistica for Windows, Release 6.0*. Samples were compared using Student's t-test.

5. Research results

In experimental animals were studied dynamics of changes in body weight (fig. 1), and the serum lipids content: total cholesterol (Hol), triglycerides (TrG) and classes of lipoproteids: high-density lipoproteins (HDLP), low (LDLP) and very low (VLDLP) density.

According to our data (fig 1), the modeling of diabetes by administering streptozotocin leads to a 15% decrease of the body weight of animals relative to Control on the 5th day of the experiment. In the Model group, which got a placebo, further significant weight loss wasn't observed. All differences in average weight in the Model and Control groups were within the statistical deviation. The introduction of the plant extract *Citrullus colocynthis* caused a significant ($p \leq 0.05$) decreasing of the body weight of all animals in 1.4 times in group 1 relative to the initial values and in 1.2 times in group 2. Thus, we found that the administration to animals with model of diabet of dry extract *Citrullus colocynthis* causes a significant weight loss of 1.2-1.4 times the initial values.

In the model of diabetes, the ratio of total cholesterol (Hol) and triglycerides (TrG) changed due to a decrease of level of the cholesterol in the Model group to 1,42±0,1 mmol/L from the control value of 1,66±0,12 mmol/L and an increase of level of triglycerides from 1,03±0,03 mmol/L in the Control group to 1,38±0,06 mmol/L in the Model group (fig. 2). Introduction to rats with a solution of *Citrullus colocynthis* with a dose of 200 mg/kg practically did not change this ratio of cholesterol and triglycerides and was in the group 1 Hol – 1,38±0,06 mmol/L and TrG – 1,25±0,11 mmol/L . Introduction of *Citrullus colocynthis* with a dose of 400 mg/kg caused an increase of cholesterol in group 2 to 1,56±0,08 mmol/l, which is not different from Control and a significants decrease of level of triglycerides to 0,77±0,06 mmol/L, even lower than Control.

The dynamics of VLDLP fluctuation was as follows: in the Model group, an increase to 0,64±0,03 mmol/L was observed, compared to the Control of 0,46±0,017 mmol/L - this was a significant difference. In group 1, VLDLP increased relative to the group with diabetes to 0,56±0,05 mmol/L, which did not differ significantly from Control, and in group 2 decreased to 0,35±0,03 mmol/L, which was statistically different from the

model and the Control group. LDLP in the observation dynamics revealed no statistically significant changes in the rats of the experimental groups. Observing the level of HDLP cholesterol showed that in rats with diabetes they decreased 2.3 times to 0,36±0,08 mmol/L in comparison with Control 0,84±0,1 mmol/L. The introduction of *Citrullus colocynthis* extract in a dose of 200 mg/kg practically did not change this ratio: in group 1 it was 0,42±0,3 mmol/L, and in group 2 the amount of HDLP was 0,86±0,8 mmol/L, then there was a rise to the level of Control.

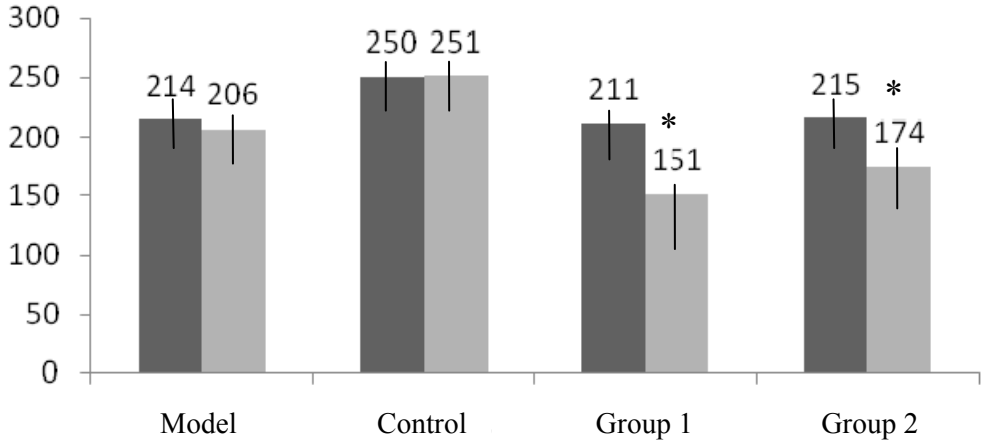

Fig. 1. Average body weight of animals (gr.) In groups of experimental rats on the 5th day of observation (dark bars) and on the 45th day of observation (light bars)
* - P≤0,05 statistically different from the baseline

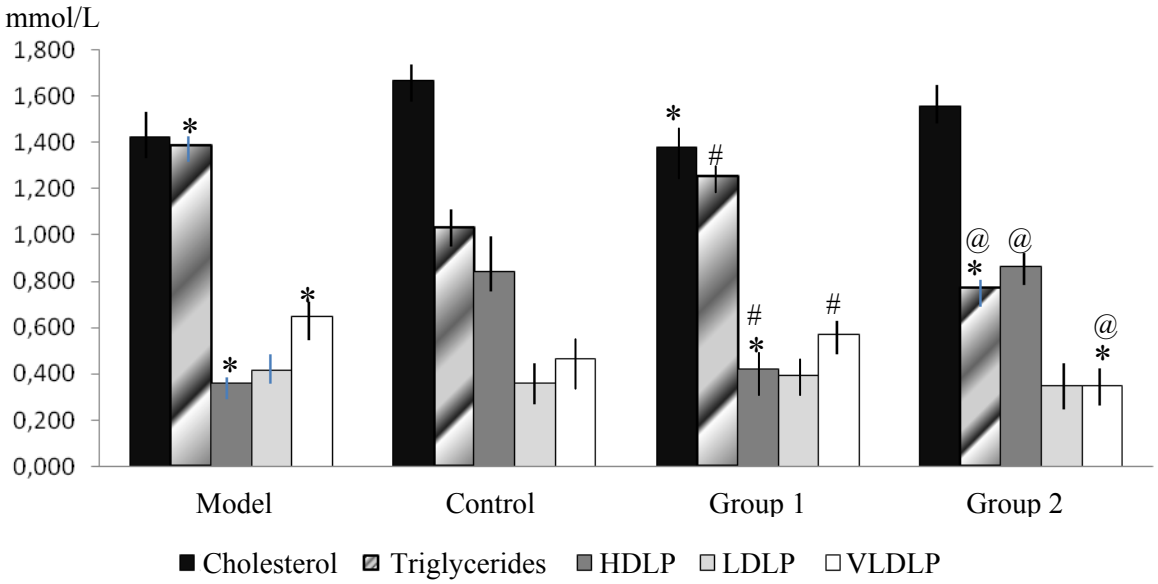

Fig. 2. Changes in lipids and classes of blood serum lipoproteins in rats of different experimental groups
* - Statistically significant difference compared to Control (p<0,05)
@ - Statistically significant difference in comparison with the diabet model (p<0,05)
# - Statistically significant difference between groups 1 and 2 (p<0,05)

Thus, we found that the introduction of dry extract *Citrullus colocynthis*, which is a source of FA, to the rats with the diabet model for 14 days, does not cause an increase in total plasma cholesterol. The effect of the *Citrullus colocynthis* plant solution 400 mg/kg caused a redistribution of the classes of

lipoproteins to the values of the control group. The dose of 200 mg/kg of the dry extract of *Citrullus colocynthis* does not cause statistically significant changes in plasma lipoproteins, but there is a positive tendency to establish a correlation of their classes.

Identification of changes in lipid metabolism under the influence of dry extract *Citrullus colocynthis* showed that the introduction of a small amount of FA into the body of an animal with type 1 diabetes can be effective and positively affect metabolic processes. Probably, not only the presence of saturated and unsaturated FA in partially defatted extract, but also their uniform distribution in raw materials, influenced the organism of experimental animals.

The study of the composition of the classes of lipoproteins was completely due to the fact that the FA is absorbed into the blood through the capillaries of the intestinal tract along with other nutrients. But the FA is too large to directly enter through small holes in the intestinal capillaries. Instead, they are absorbed by villi in the intestinal wall and synthesized into triglycerides. Triglycerides are coated with cholesterol and proteins to form chylomicron. In cells of peripheral organs and tissues, cholesterol exists mainly in the form of low density lipoproteins (LDLP) and in the form of phospholipids. Phospholipids are used by cells to build or renew their membranes. Cholesterol, which comes in the composition of LDLP, can also be used in cells to build membranes. Excess cholesterol in cell membranes disrupts their viscosity and the work of transmembrane transport systems.

High density lipoproteins (HDLPs) are particles that capture excess cholesterol from peripheral tissue cell membranes and transport it to either the liver or the intestines, that is, the functioning of HDLP cholesterol helps remove excess cholesterol from the body.

6. Conclusions

Introduction to rats with type 1 diabetes of the extract of a partially defatted *Citrullus colocynthis* extract with a dose of 400 mg/kg for 14 days causes a redistribution of classes of lipoproteins to the values of the control group. The dose of 200 mg/kg of the dry extract of *Citrullus colocynthis* does not cause statistically significant changes in plasma lipoproteins, but there is a positive tendency to establish a correlation of their classes.

Thus, *Citrullus colocynthis* extract, which contains micro doses of FA in their uniform distribution, has a positive effect on lipid metabolism, does not contribute to an increase in total cholesterol, does not cause an increase of the body weight.

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Вплив мікродоз жирних кислот екстракту *citrullus colocynthis* на ліпопротеїди крові щурів з цукровим діабетом 1 типу

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Мета: Дослідити вплив мікродоз жирних кислот (ЖК) частково знежиреного сухого екстракту *Citrullus colocynthis* на ліпідний обмін щурів з цукровим діабетом (ЦД) 1 типу. **Методи дослідження:** Сировину знежирювали шляхом екстракції в апараті Сокслета за умов скорочення часу випарювання. За допомогою газорідинного хроматографічного дослідження виявлено у складі отриманого сухого екстракту *Citrullus colocynthis* слідові концентрації ЖК. **Результати:** Встановлено, що введення щурам екстракту рослинного розчину *Citrullus colocynthis* в дозі 400 мг/кг викликає перерозподіл класів ліпопротеїдів до значень контрольної групи. Доза 200 мг/кг – не викликає статистично достовірних змін ліпопротеїдів плазми крові. **Висновки:** Узагальнено, що екстракт *Citrullus colocynthis* який містить мікродози ЖК при їх рівномірному розподілу позитивно впливає на ліпідний обмін, не сприяє підвищенню загального холестерину, не викликає збільшення маси тіла.

Ключові слова: жирні кислоти; ліпопротеїди; тригліцериди; холестерин; цукровий діабет.

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Влияние микродоз жирных кислот экстракта *citrullus colocynthis* на липопротеиды крови крыс с сахарным диабетом 1 типа

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Цель: Исследовать влияние микродоз жирных кислот (ЖК) частично обезжиренного сухого экстракта *Citrullus colocynthis* на липидный обмен крыс с сахарным диабетом (СД) 1 типа. **Методы исследования:** Сырье обезжиривали путем экстракции в аппарате Сокслета в условиях сокращения времени выпаривания. С помощью газожидкостного хроматографического исследования выявлено в составе полученного сухого экстракта *Citrullus colocynthis* следовые концентрации ЖК. **Результаты:** Установлено, что введение крысам экстракта растительного раствора *Citrullus colocynthis* дозой 400 мг/кг вызывает перераспределение классов липопротеидов до значений контрольной группы. Доза 200 мг/кг – не вызывает статистически достоверных изменений липопротеидов плазмы крови. **Выводы:** Обобщено, что экстракт *Citrullus colocynthis* содержащий микродозы ЖК при их равномерном распределении положительно влияет на липидный обмен, не способствует повышению общего холестерина, не вызывает увеличения массы тела.

Ключевые слова: жирные кислоты; липопротеиды; сахарный диабет; триглицериды; холестерин.

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