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INFLUENCE OF *CITRULLUS COLOCYNTHIS* (L.) SHRAD DRY FRUITS EXTRACT ON PANCREAS β-CELLS VIABILITY

The study investigated the ability of Citrullus colocynthis dry fruits extract to modulate survival of RIN-m5F cells in vitro. Various concentrations of Citrullus colocynthis had no toxic effect on the cells viability. Exposure of β-cells to high glucose concentration and prooxidant agents like tert-butylhydoperoxide and oleic acid had cytotoxic effects on their viability. Citrullus colocynthis protected cells against investigated agents action. Positive effect of Citrullus colocynthis could be associated with its direct modulatory action on regeneration and functional normalization of pancreas β-cells or/and indirectly through metabolic and antioxidant effects of Citrullus colocynthis constituents. Our findings provide important feasible data for utilizing Citrullus colocynthis for treatment of diabetes mellitus.

Key words: Citrullus colocynthis (L.); diabetes mellitus; β-cells viability

INTRODUCTION

For diabetes mellitus (DM), a multifactorial disease characterized by a relative or absolute lack of insulin or resistance to its action at the cellular level [15]. Interraption of glucose metabolism in the body leads to dysfunction of β-cells of the pancreas, accompanied by their death as well as the occurrence of insulin resistance. Under such conditions, considerable changes in the metabolism of lipids and proteins [4, 12, 24].

Despite the wide range of pharmacological antidiabetic drugs, which are used in clinical diabetes and its complications, currently an increasing emphasis on the use of herbal medicine that is based on the use of plants as well as preventive and drugs [17, 25]. Plants as sources of phytotherapeutic drugs have found use in the treatment of diseases, including diabetes, but the mechanisms underlying their therapeutic action, and the most effective active ingredient, a part of them finally clarified.

However, we know that plants that are used for treatment contain alkaloids, and quite often different alkaloids are part of the same plants that promotes the use of natural sources as raw materials for development and a more effective modern treatments, especially when the content of the active ingredient, which is part of their content much higher than other components [11, 17].

Currently numbering more than 700 species of medicinal plants that possess hypoglycemic action and are used in clinical diabetes [1, 19]. Thus, by dietary components of *Galega officinalis L.* enhanced glucose uptake by skeletal muscles of rats, regeneration of β-cells of the Langerhans islets, etc. [9, 21]. Different parts of the plant (aerial part of grass, seeds, roots) contain different amounts of active ingredients, especially alkaloids preferred in hypoglycemic action halehy drug [20] because of its alkaloid (halehin) in its chemical structure and pharmacological activity is analogous antidiabetic biguanide drugs row (hlibutyd, metformin etc.) that are currently used in the treatment of type 2 diabetes.

Among the herbs attention of researchers and physicians focused on the use of clinical diabetes *Citrullus colocynthis* (L.) Shrad. This pumpkin "colocynth" which is a perennial herb that belongs to the family Pumpkin (Cucurbitaceae) and distributed in Africa, India, Iran and the Mediterranean. *Citrullus colocynthis* been as an antibacterial, antimalarial, antidiabetic, anticancer, antihelmintic agent [7, 14]. An extremely wide range of action due to the fact that the composition of the fruit are colocynthyn, colocyntein, colocyntetin, kukurbitacyn E 2-O-beta-D-glucoside, pectin, etc. [6, 8]. From literary sources we know that pumpkin "colocynth" has antydiabetic action, including extracts from its leaves [10]. Extract from other parts of the plant are effective for experimental streptozotocin-induced diabetes and other toxic compounds [23] and its application in the treatment of patients with diabetes [13]. Despite the data, the mechanisms involved in the action components of fruits *Citrullus colocynthis* finally unknown.

Based on the above, the purpose of this study was to find out whether the *Citrullus colocynthis* (extract of dried fruits) cytoprotective effect on the cell line RIN-m5F for the effect of high glucose and oxidative factors.

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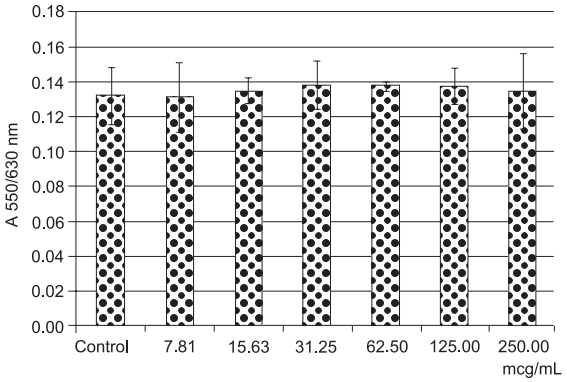

Fig. 1. *Effect of extract powder from dry fruits Citrullus colocynthis on the viability of cells RIN-m5F, M ± m.*

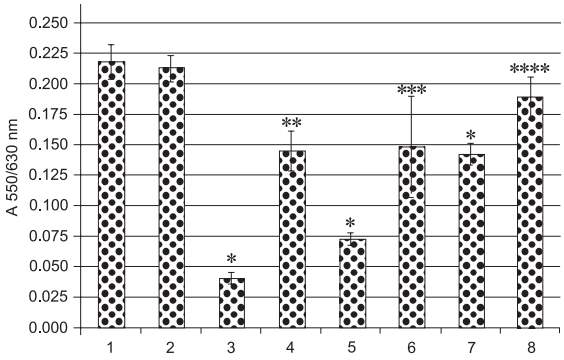

Fig. 2. *Effect of extract from powder dry fruits Citrullus colocynthis at a concentration of 125 mg/ml on cell viability RIN-m5F for the actions of various factors, M ± m.*

Legend: 1 – control; 2 – *Citrullus colocynthis*; 3 – glucose 111 mmol/l; 4 – glucose + *Citrullus colocynthis*; 5 – t-butylhydroperoxyd (tBHP) 25 mmol/l; 6 – t-butylhydroperoxyd + *Citrullus colocynthis*; 7 – oleic acid 50 mmol/l; 8 – oleic acid + *Citrullus colocynthis*.

Notes: * – p < 0.05 relative to control; ** – P < 0.05 relative influence of glucose into cells; *** – P < 0.05 relative impact on butylhydroperoxyd t-cells; **** – P < 0.05 relative to the impact of oleic acid in cells.

MATERIALS AND METHODS

Dry powder fruit of *Citrullus colocynthis* after degreasing received at the Department of Pharmacognosy and Botany of the Bogomolets National Medical University according to the method [1]. Evaluation of biological effects of extract powder dry fruits *Citrullus colocynthis* on the cell line RIN-m5F (β-cells in the pancreas of rats) conducted using MTT test, as described [18], in their own modifications. The method is based on the reduction reaction cells in culture tetrazolum dye MTT (3,4,5-dymetyltiazol 2.5 dyfeniltetrazolium bromide) to MTT-formazan, whose intensity reflects the degree of cell viability as a result of the restoration of dye by mitochondrial and partially cytoplasmic dehydrogenases. For this target cells in a concentration 2.5h105 cells/ml made in 96 well plates wells (Greiner, Germany) in a volume of 100 ml suspension in RPMI-1640 medium with the addition of 1 mmol/L sodium pyruvate, a mixture of antibiotics: 100 IU/ml penicillin, 100 ug/ml streptomycin, 0.25 mg/ml amphotericin B, 10 % fetal bovine serum and cultured for 24 hours under standard conditions (5 % CO_2, 100 % humidity, 37 °C). Dry fruits of *Citrullus colocynthis* powder dissolved in sodium phosphate buffer PBS (Eng. Phosphate buffered saline, pH 7.4), passed through a 0.22 micron filter (Millipore, USA) and prepared a series of dilutions. Before analysis, the incubation medium was replaced for new (no fetal bovine serum) was added and samples. Then cultured for 24 hours under standard conditions. Later, carefully selected incubation medium added to 100 ml MTT solution at a concentration of 1 mg/ml in PBS buffer and cultured for 4 hours at 37 °C. At the end of incubation, MTT solution selected was added 100 ml solution of DMSO and incubated for 5 minutes, under conditions of intense shaking. Optical absorption solution was measured at a wavelength of 550 nm (research) and 630 nm (assistant) on a spectrophotometer BioTech uQuant (BioTek Instruments Inc, USA). All samples analyzed in triplicate. Processing of the data was performed program Microsoft Exscel 2010. Results presented as the mean (M) and the stundart deviation (± m). The difference between the medium group was considered statistically significant at P < 0,05.

RESULTS AND DISCUSSION

In our studies previously conducted on an experimental model of type 1 diabetes induced by streptozotocin determind of functioning islet cells of the pancreas [2]. However, in another experimental model of diabetes type 1, we shown that an extract from dried fruits *Citrullus Colocynthis* has hypoglycemic action [3].

In order to determine whether hypoglycemic effect of *Citrullus Colocynthis* fruit at the expense of its action directly on β-cells of the pancreas or indirectly through the impact on other target tissue, initially it was important to ensure that the investigational drug with dry fruits *Citrullus Colocynthis* has no toxic effect on the viability of the cell line RIN-m5F. Despite the fact that different parts of *Citrullus colocynthis* such as roots, fruits, bark, seeds and leaves are used to prepare extracts for treatment, and for animals used doses within 10-500 mg per kg of body weight per day [22] we focused on the study of dry powder fruit *Citrullus colocynthis*. The effect of the dry powder from the fruit Citrullus Colocynthis studied in a wide range of concentrations, namely in the range: 7.8-250 mg/ml. According to the results of MTT test application *Citrullus Colocynthis* in all studied concentrations did not affect the viability of cells studied, suggesting that it is not cytotoxic to β-cells of the pancreas (Fig. 1). However, given the limited solubility of dry powder *Citrullus Colocynthis* in further studies used in a final concentration of 125 mcg/mL.

It is known that in diabetes there are significant functional and metabolic disorders, as a result of hyperglycemia and oxidative stress, which leads to the intensification of violations of the structure of cell membranes [26]. In this regard, it was important to assess the effect of oxidative factors on cells of the pancreas and deter-

mine whether the components have hypoglycemic fruits of *Citrullus Colocynthis*, antioxidant and/or cytoprotective effect for the duration of 24 hours of incubation.

In the study of the high concentration of glucose 111.0 mg/dL, the conditions for mimikuyut hyperglycemia of diabetes, the viability of target cells significantly decreased, while introduction of *Citrullus Colocynthis* in the incubation medium prevented cell death, as evidenced by the increase in the number of living cells 48 1 % (Fig. 2). Under conditions of oxidative stress induced by different chemical nature of the factors that have found use in cell culture studies [16], such as t-butylhydroperoxydom (25.0 mmol/L) and oleic acid (50.0 mmol/l) content living cells decreased by 66.7 % and 34.7 %, respectively (Fig. 2). Use dry powder extract from *Citrullus Colocynthis* fruit at a concentration of 125 mcg/ml against a background of prooxidant factors led to a decline in their destructive action on the cells of the pancreas, it is possible that due to the antioxidant properties of the components of the fruit, as the content of living cells increased by 34.8 % and 21.1 % respectively compared with the effect of t-butylhydroperoxyd and oleic acid.

CONCLUSIONS

In cell line RINm5F found that the hypoglycemic mechanisms, cytoprotective and antioxidant action of the extract powder from dry fruits *Citrullus Colocynthis* on the cell line RIN-m5F implemented by cumulative impact of dietary components that it contains. Moreover, we found that one of the main targets of positive pharmacological action of an extract from the fruit dry powder *Citrullus Colocynthis* in the treatment of diabetes can be just β-cells of the pancreas.

Thus, the data not only complement existing information on the mechanisms of pharmacological action of dry powdered fruit of Citrullus Colocynthis, but also to justify the appropriateness of its use in the treatment of diabetes and its many complications. These studies also contribute to the search and development of new effective drugs, to which he will include them or be the basis for diabetes, it is possible that by promoting regeneration and recovery of function β-cells of the pancreas, preventing their death. However, it should be noted that the mechanism of destruction of β-cells is extremely difficult, because finding out the launch and progress of all pathogenic processes require further research.

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ВЛИЯНИЕ ЭКСТРАКТА СУХИХ ПЛОДОВ *CITRULLUS COLOCYNTHIS* (L.) SHRAD НА ЖИЗНЕСПОСОБНОСТЬ β-КЛЕТОК ПОДЖЕЛУДОЧНОЙ ЖЕЛЕЗЫ

Исследована способность экстракта сухих плодов *Citrullus colocynthis* влиять на выживаемость клеток RIN-m5F *in vitro*. Различные концентрации *Citrullus colocynthis* не оказывали токсического действия на жизнеспособность клеток. Воздействие на клетки высокой концентрации глюкозы и прооксидантных агентов, таких как трет-бутилгидропероксид и олеиновая кислота вызывало цитотоксическое действие на жизнеспособность β-клеток. *Citrullus colocynthis* оказывал защитный эффект против действия исследуемых агентов. Положительный эффект *Citrullus colocynthis* может быть ассоциирован с его непосредственным влиянием на регенерацию и нормализацию функций β-клеток поджелудочной железы и/или косвенно посредством метаболических и антиоксидантных свойств составляющих *Citrullus colocynthis*. Наши исследования свидетельствуют о возможности использования *Citrullus colocynthis* для лечения сахарного диабета.

Ключевые слова: *Citrullus colocynthis (L.)*; сахарный диабет; жизнеспособность β-клеток

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ВПЛИВ ЕКСТРАКТУ З СУХИХ ПЛОДІВ *CITRULLUS COLOCYNTHIS* (L.) SHRAD НА ЖИТТЄЗДАТНІСТЬ β-КЛІТИН ПІДШЛУНКОВОЇ ЗАЛОЗИ

Досліджено здатність екстракту сухих плодів *Citrullus colocynthis* впливати на виживання клітин RIN-m5F *in vitro*. Різні концентрації *Citrullus colocynthis* не чинили токсичної дії на життєздатність β-клітин. Вплив на β-клітини високої концентрації глюкози і прооксидантних агентів, таких як трет-бутилгідропероксид і олеїнова кислота викликав цитотоксичну дію на їх життєздатність. *Citrullus colocynthis* володів захисним ефектом проти дії досліджуваних прооксидантів. Позитивний ефект *Citrullus colocynthis* може бути асоційований з його безпосереднім впливом на регенерацію і нормалізацію функцій β-клітин підшлункової залози і/або опосередковано через метаболічні та антиоксидантні властивості складових *Citrullus colocynthis.* Наші дослідження свідчать про можливість використання *Citrullus colocynthis* для лікування цукрового діабету.

Ключові слова: *Citrullus colocynthis* (L.); цукровий діабет; життєздатність β-клітин

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