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YACON'S (*SMALLANTHUS SONCHIFOLIUS* POEPP. & ENDL.) EFFECTS ON POSTPRANDIAL GLUCOSE UNDER EXPERIMENTAL DIABETES MELLITUS

The article describes the finding of the research yacon leaves and root tubers water extracts as well as yacon root tubers suspensions effects on glucose tolerance in animals with experimental diabetes mellitus. It has been shown that the studied yacon extracts and suspensions at the dose of 0.5 g/kg produce significant hypoglycemic effect under the experimental diabetes mellitus. The glucose tolerance test and the analysis of the area under the glycemic curves have revealed that yacon root tubers in the form of a stabilized suspension exert the best hypoglycemic effect.

Key words: yacon (*Smallanthus sonchifolius* Poepp. & Endl.); diabetes mellitus; hypoglycemic effect

INTRODUCTION

Epidemiological and clinical studies have revealed that exogenously-induced postprandial hyperglycemia affects the development of diabetes mellitus, as well as increases the risk of its complications [3].

In recent years functional food intake has raised significantly throughout Europe, North and South Americas, Japan and other countries. Such foods have a considerable potential for the food industry. They are essential for food structural improvements, for maintaining health and for prevention of modern civilization diseases such as atherosclerosis, obesity, oncological diseases, osteoporosis and diabetes mellitus (DM) [12].

Functional foods contain components that can be eliminated, enriched or substituted in nutrients (macro- and micronutrients) and biologically active substances. Foods are considered functional if they are enriched by dietary fibers, prebiotics and probiotics of microorganisms (*Bifidobacterium* and *Lactobacillus*), antioxidants, vitamins (A, E, C, beta-carotene), minerals (calcium etc.), microelements (iron, zinc, fluorine, selenium etc) and flavonoids (phytoestrogens, quercetin etc.) [8].

In functional foods, special attention is devoted to inulin-containing medicinal plants, which are increasingly used in the diet of persons with diabetes. Inulin is an polysaccharide of root tubers, which consists of D-fructofuranose residues linked by β-2,1-glycosidic bonds. The polysaccharide chain ends with α-D-glucopyranose residue. As for dietary properties of inulin, they are caused by that β-2,1-glycoside linkage. Since human enzymes are not capable of cleaving the bond, inulin remains intact while going through almost the whole gastrointestinal tract up to the large intestine where it is cleaved by *Bifidobacterium* enzymes.

Inulin is a soluble dietary fiber that inhibits the rate of nutrients absorption, thus promoting more equal insulin secretion after food consumption and, accordingly, more gradual increases and decreases in the level of blood sugar [18].

Yacon (*Smallanthus sonchifolius* Poepp. & Endl. or *Polymnia sonchifolia* Poepp. & Endl.), an inulin-containing plant, is a potential source of functional foods. It has been recorded in the literature that water extracts of yacon leaves have been used for centuries by the indigenous people of the Andes for DM treatment. In modern medicine, the fact that biologically active substances of yacon leaves reduce the level of peripheral blood glucose was first revealed in 1997 [9]. Later on, in the 21-st century, the hypoglycemic effect of the water extract of yacon leaves was proved by findings on the model of type 1 experimental diabetes mellitus (EDM) [1, 9].

The overall aim of the present study was to search the effects of the water extract of yacon leaves, the extract and suspension of yacon root tubers on glucose tolerance under type 1 experimental diabetes mellitus.

MATERIALS AND METHODS

Experimental Animals

The experimental study has been carried out on Wistar rats with the weight of 130-180 g in accordance with general ethical standards for experiments performed on animals laid down by the First National Congress on Bioethics (Kyiv, Ukraine, 2001), which conform to the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes (Strasbourg, France, 1985) and the Law of Ukraine on protection of animals against cruel treatment (dated 26 February 2006).

The following groups of animals were used in the experiment: 1) control animals (**C**); 2) control animals that were treated orally with water extract of yacon leaves at doses 0.07 g/kg /day (**C + E_L^{70}**) and 0.5 g/kg / day (**C + E_L^{500}**); 3) control animals that were treated orally with water extract of yacon root tubers at doses 0.07 g/kg /day (**C + E_R^{70}**) and 0.5 g/kg / day (**C + E_R^{500}**); 4) control animals that were treated orally with water suspension of root tubers powder (**C + S**) and with water suspension of root tubers powder stabilized by biocomplex PS (**C + S^{PS}**); 5) animals with experimental diabetes mellitus (**DM**); 6) animals with diabetes that were treated orally with yacon leaves water extract at doses 0.07 g/kg /day (**DM + E_L^{70}**) and 0.5 g/kg /day (**DM + E_L^{500}**); 7) animals with diabetes that were treated orally with yacon root tubers water extract at doses 0.7 g/kg /day (**DM + E_R^{70}**) and 0.5 g/kg /day (**DM + E_R^{500}**); 8) animals with diabetes that were treated orally with water suspension of root tubers powder (**DM + S**) and with water suspension of root tubers powder stabilized by biocomplex PS (**DM + S^{PS}**).

Induction of Diabetes

Type 1 EDM was induced by an intraabdominal injection of streptozotocin ("Sigma", the USA) in the dose of 0.055 g per 100 g of body weight. Glucose concentrations in blood were measured by the electrochemical glucometer. The animals used in the experiment had glucose levels of 14 mmol/l and higher (after going without food for 18 hours). Yacon water extracts or suspensions were administered to the animals *per os* in two weeks after the induction of EDM.

Preparation of Water Extracts

During the first stage, yacon leaves and root tubers were used in the experiment to produce water extracts by infusion of certain parts of the plant (the ratio was 1:10) in two consecutive phases: 15 min at 100 °C and 45 min at 20 °C. The extracts were filtered and vaporized in the vacuum to receive dry remnant by LABOROTA 4001 rotor vaporizer (Heidolph, Germany) at 60-65 °C. The vaporized yacon extract infusions were administered to the animals *per os* in the dose of 0.07 g/kg and 0.5 g/kg of body weight for 14 days.

Preparation of Water Suspension of Root Tubers Powder

Suspension made of yacon root tubers was used during the second stage of the experiment. It was received by mixing homogeneous powdered root tubers with water. Surface-active substances of biogenic origin (biocomplex PS) in the concentration of 0.1 ml per 1 ml of the suspension were added to the suspension to increase its stability and bioavailability of biologically active substances. Surface-active biocomplex PS was collected from the supernatant of the culture liquid of Pseudomonas sp. PS-17 strain. The obtained suspension and its stabilized form were administered to the animals *per os* in the dose of 0.5 g/kg of body weight for 14 days.

Glucose Tolerance Test Assay

The glucose tolerance test was performed in the morning after an 18-hour fasting of the animals. Glucose levels were determined from the blood taken from rat tail veins before and after the hydrocarbon load. The graph, a glycemic curve, was constructed based on the obtained results. It shows the rate of glucose assimilation and how the introduction of yacon extracts and suspensions affects the level of blood sugar (the null point is on an empty stomach, in 10, 20, 30, 40, 50 and 60 min after glucose taking).

The calculated integral index of the area under the glycemic curve (*AUCglu*), which shows a general increase in glucose concentrations after glucose consumption, served as the criterion for the total response to the standard glucose tolerance test. The area under the glycemic curve was calculated by the trapezoid rule [17].

Statistical Analysis

Data are expressed as mean ± S.E.M. Differences among experimental groups were determined by ANOVA (analysis of variance), and the significance of between-group differences assessed by Student-Newman-Keul's multiple range test. Significance was defined at $P \leq 0.05$.

RESULTS AND DISCUSSION

Previously performed screenings had revealed significant effects of yacon water extracts made from petioles, stems, leaves, root tubers and root tubers peels on glucose tolerance in healthy animals in the dose of 0.07 g/kg of body weight [10]. Considering obtained results, we decided to conduct a study of influence of extracts with most prominent hypoglycemic effect on the efficiency of carbohydrates digestion under the condition of EDM.

The glucose tolerance test is accurate for clinical diagnosis of diabetes mellitus since it checks the rate of glucose assimilation in the organism and establishes disturbances in this process [12].

It has been revealed that glycemic curves were considerably changed in animals with EDM. It was established a dramatic increase in glucose concentrations after glucose loading in their blood on the 10-th minute and a further increase on the 20-th min of the experiment. As for control animals, the maximal glucose concentration was on the 20-th min and a full return to the initial level of glucose was noted on the 30-th min of the experiment, whereas under EDM glucose concentrations were back to normal on the 50-th min (Fig. 1 A). Such changes in glycemic curves under EDM caused a 2.42 times increase in the area under the glycemic curve, which testifies the disturbances in the efficiency of glucose assimilation by tissues under the studied pathology (Fig. 1 B).

When animals with EDM were treated with yacon leaves water extract in the dose of 70 and 0.5 g/kg, the initial level of glucose decreased correspondingly by 11.65 % and 40.05 %. After glucose loading and when the extract was administered in the dose of 0.7 g/kg, there was a slight increase in the level of glucose on the 10-th min followed by its dramatic decrease to the initial values.

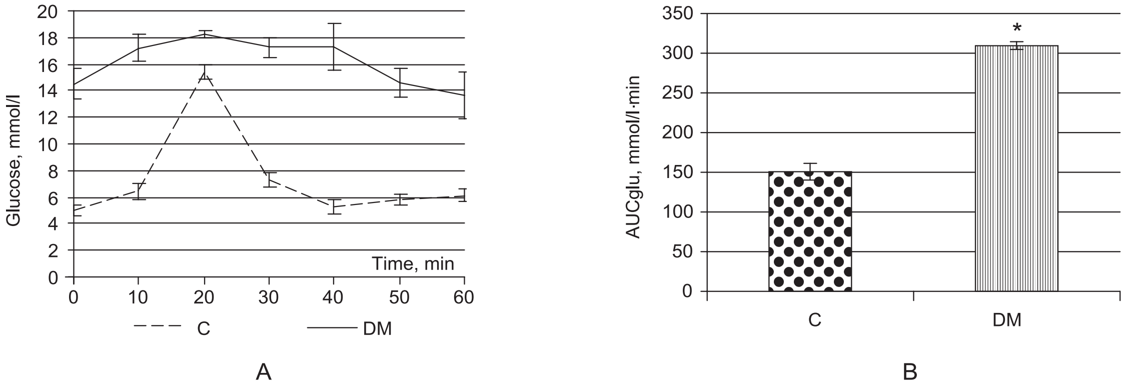

Fig. 1. A) *Changes in the glucose concentration in the blood of healthy animals and animals with EDM after carbohydrate loading.* **B)** *The area under the glycemic curves of healthy animals and animals with EDM (M ± m, n = 4-8): *P < 0.05 compared with controls; #P < 0.05 compared with diabetic rats without water extracts or water suspension consumption; *#P < 0.05 compared with the control and diabetic rats without water extracts or water suspension consumption*

When the extract was administered in the dose of 0.5 g/kg, there was no peak of glucose concentration and its level was almost the same as the basal one for an hour (Fig. 2 A). It also caused a decrease in AUCglu, compared with EDM, by 44.11 %. On the other hand, the dose of 0.7 g/kg did not produce such an effect (Fig. 2 B). Therefore, yacon leaves water extract in the dose of 0.5 g/kg of body weight brings more significant hypoglycemic effect under the condition of EDM.

The hypoglycemic effect of yacon leaves may be attributed to phenols in their composition such as caffeic, chlorogenic, ferulic and protocatechuic acids. Phenols inhibit alpha-amylase and sucrase, depress the delivery of glucose to the cells of the gastrointestinal tract by inhibiting sodium-glucose transporter-1 (S-GLUT-1). It is known that chlorogenic acid inhibits glucose-6-phosphotase, thus affecting metabolism of hydrocarbons (glycolysis, glycogenolysis and gluconeogenesis) [9]. Another active component of yacon leaves is enhydrin, sesquiterpene lactone, which increases the number of β cells in pancreatic islets of rats with streptozotocin-induced DM as well as the level of insulin mRNA of those cells [2, 9, 15]. The synergistic action of all the compounds leads to the marked hypoglycemic effect.

The glycemic peak decreased on the 20-th min due to the introduction of yacon leaves water extract in the examined concentrations to control animals (Fig. 3 A). Despite changes in glycemic curves, *AUCglu* index remained the same as control values (Fig. 3 B).

Compared with EDM, the introduction of yacon root tubers water extract in the doses of 0.7 and 0.5 g/kg caused a decrease in the initial level of glucose by 28.47 % and 54.04 %. Moreover, the extract in the examined concentrations led to unidirectional changes in the character of glycemic curves. It was estimated an increase in glucose concentration on the 20-th min after the hydrocarbon loading followed by its gradual decrease on the 30-th min. The level of glucose was reaching its initial value on the

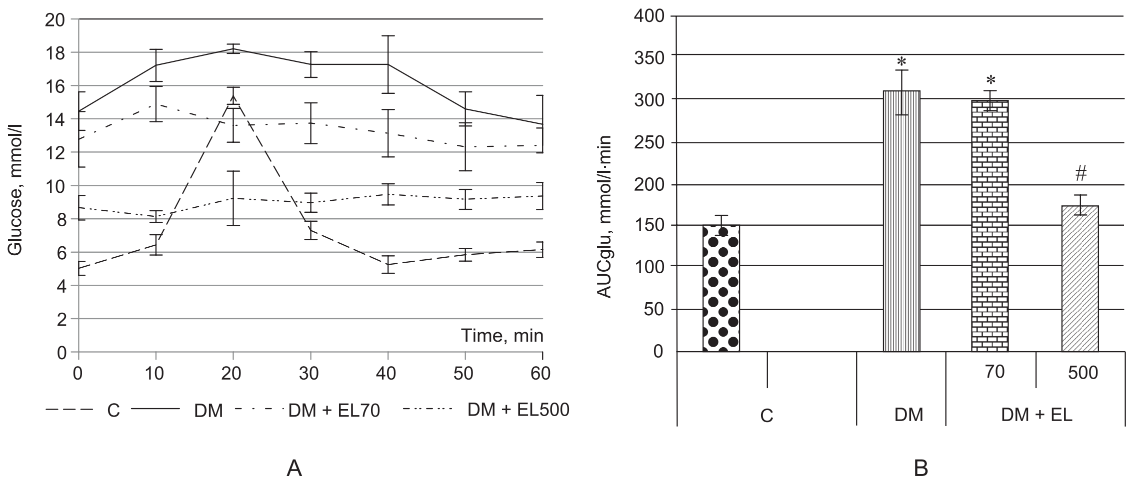

Fig. 2. A) *Effect of yacons leaves water extract at doses 0.7 and 0.5 g/kg on blood glucose concentration changes of diabetic rats.* **B)** *The area under the glycemic curves in animals with EDM that were treated with the water extracts of yacons leaves (0.7 and 0.5 g/kg) (M ± m, n = 4-8)*

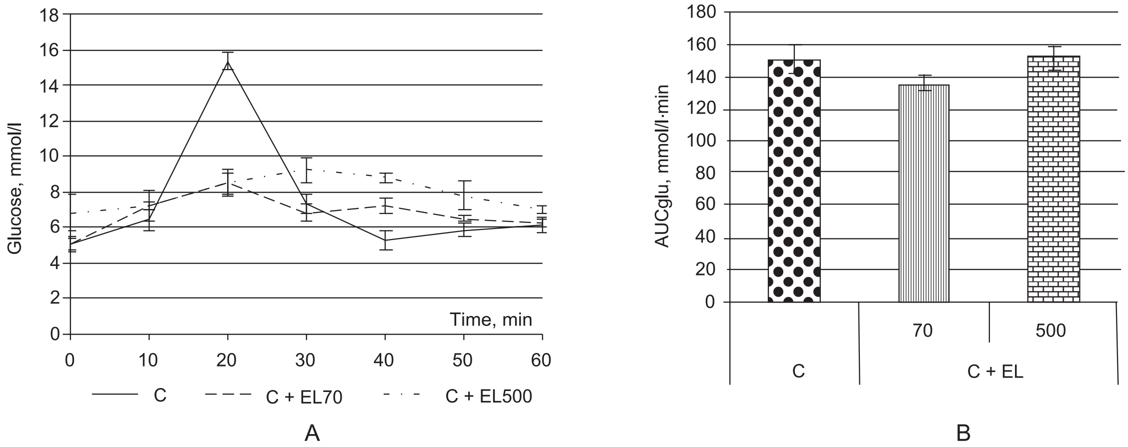

Fig. 3. A) *Effect of yacons leaves water extract at doses 0.7 and 0.5 g/kg on blood glucose concentration changes of healthy rats.* **B)** *The area under the glycemic curves in healthy animals that were treated with the water extracts of yacons leaves (0.7 and 0.5 g/kg) (M ± m, n = 4-8)*

50-th min of the experiment (Fig. 4 A). As for the yacon root tuber water extract in the dose of 500 mg/kg, it exerted more significant hypoglycemic effect, which was testified by a decrease in AUCglu index by 34.12 %. On the other hand, when the extract was in its lower dosage, the area under the glycemic curve was smaller by 24.80 %, compared with the analogous index in animals with EDM (Fig. 4 B).

While researching into the hypoglycemic effect of the yacon root tubers extract in the examined concentrations, it was revealed that it produces a marked effect on glucose tolerance in healthy animals. The character of the glycemic curve changed when the extract was introduced in the dose of 0.7 g/kg – its peak fell and shifted to the 30-th min. In the dose of 0.5 g/kg, the peak was less marked (20-th min) and the repeated increase in glucose concentration occurred on the 40-th min after the hydrocarbon loading (Fig. 5 A). The shift and splitting of the peak may be attributed to inhibition or slowing-down of glucose assimilation in the gastrointestinal tract under the action of biologically active substances of yacon root tubers. A decrease in *AUCglu* (by 32.74 %, compared with control) when treated with the dose of 0.7 g/kg also testified the changes in the hydrocarbon assimilation (Fig. 5 B).

Water was chosen for extraction since it dissolves and extracts hydrophilic inorganic and organic compounds better than other solvents. Apart from that, water is a pharmacologically indifferent, non-flammable, nonexplosive and, equally importantly, affordable extractant. However, water has its drawbacks. In particular, it does not dissolve and, correspondingly, does not extract hydrophobic substances. It does not possess antiseptic properties and, as a result, microorganisms may be developed in water extracts. Hydrolytic decomposition of various substances occurs owing to water, especially at high temperatures. In addition to that, medicinal substances can be decomposed by enzymes in water [4]. Therefore, we have chosen to administer the plant in the form of suspension.

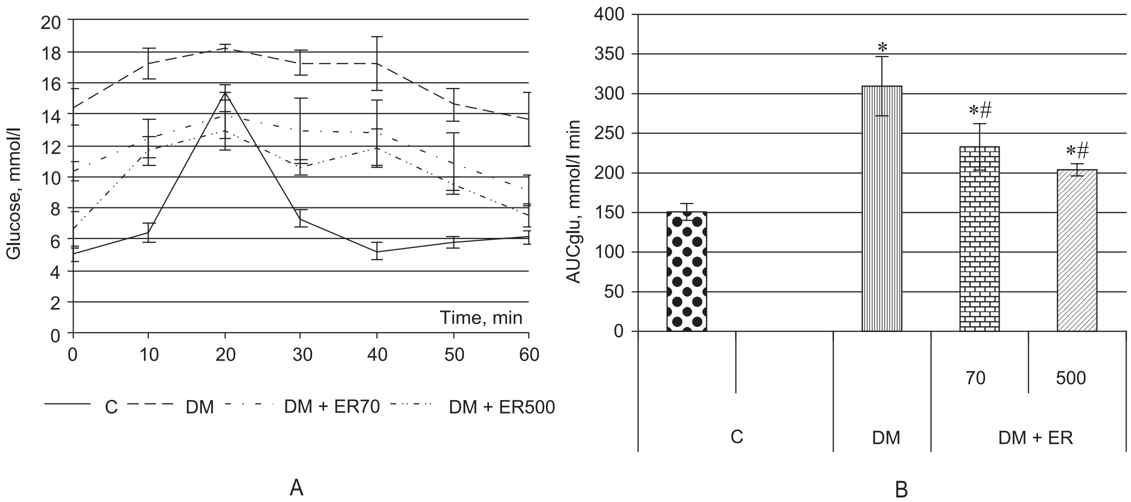

Fig. 4. A) *Effect of yacons root tubers water extract at doses 0.7 and 0.5 g/kg on blood glucose concentration changes of diabetic rats.* **B)** *The area under the glycemic curves in animals with EDM that were treated with water extract of yacons root tubers (0.7 and 0.5 g/kg) (M ± m, n = 4-8)*

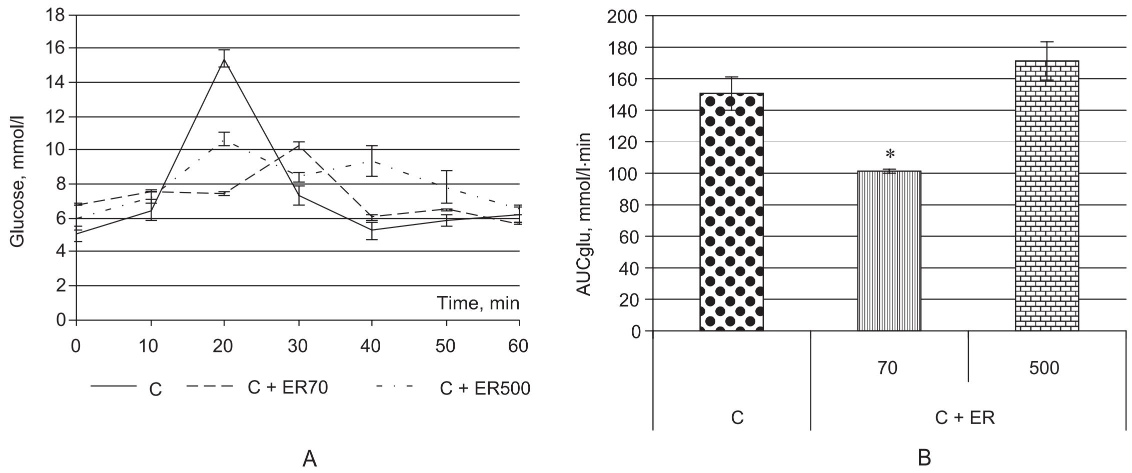

Fig. 5. A) *Effect of yacons root tubers water extract at doses 0.7 and 0.5 g/kg on blood glucose concentration changes of healthy rats.* **B)** *The area under the glycemic curves in healthy animals that were treated with water extracts of yacons root tubers (0.7 and 0.5 g/kg) (M ± m, n = 4-8)*

From the biopharmaceutical point of view, suspension has a number of advantages. For example, suspension makes it possible to produce medicines of prolonged action (a depository of medicinal substances), to regulate the duration of their action by changes in the size of medicinal raw materials particles, to use simultaneously both soluble and insoluble medicinal substances. Suspension allows wider variations in consumer properties by means of corrective substances, which mask unpleasant taste and smell of medicines. Moreover, a number of medicinal substances display the most marked coating action when used in suspension [6].

The study on glucose loading has revealed that both forms of yacon root tubers suspensions (stabilized by biocomplex PS and non-stabilized) cause a considerable decrease in the initial level of glucose, correspondingly, by 39.30 % and 40.13 %. After glucose loading, when treated with a non-stabilized suspension, there was an increase of glucose level on the 10-th and 20-th min with further decrease on the 30-th min and return to the initial level already on the 40-th min of the experiment. On the other hand, a stabilized suspension with glucose loading led to a slight increase in glucose concentrations on the 10-th min. On the 20-th-50-th min, the glucose level was within the basal value. It should be noted that on the 60-th min of the experiment the sugar level in blood was lower than at the beginning of the glucose loading. Compared with EDM, AUCglu index decreased, correspondingly, by 43.21 % and 47.04 % as a result of changes in glycemic curves following the administration of the non-stabilized and stabilized suspensions (Fig. 6).

Suspensions made of yacon root tubers exerted a marked hypoglycemic effect on the control groups of animals. When such suspensions were administered following glucose loading, there were no considerable fluctuations in glucose concentrations. The administration of both non-

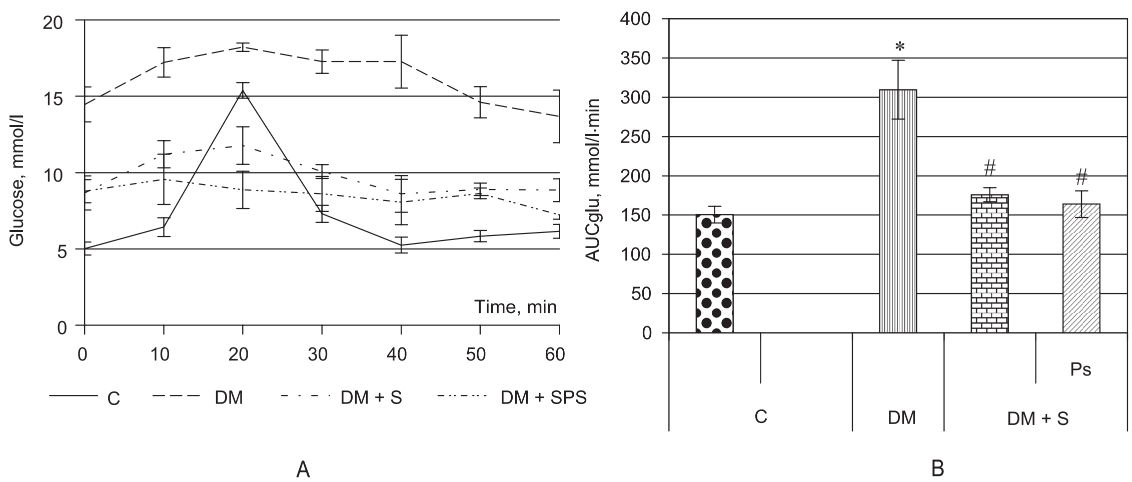

Fig. 6. A) *Effect of yacons root tubers water suspensions on blood glucose concentration changes of diabetic rats.* **B)** *The area under the glycemic curves in animals with EDM that were treated with the water suspensions of yacons root tubers (M ± m, n = 4-8)*

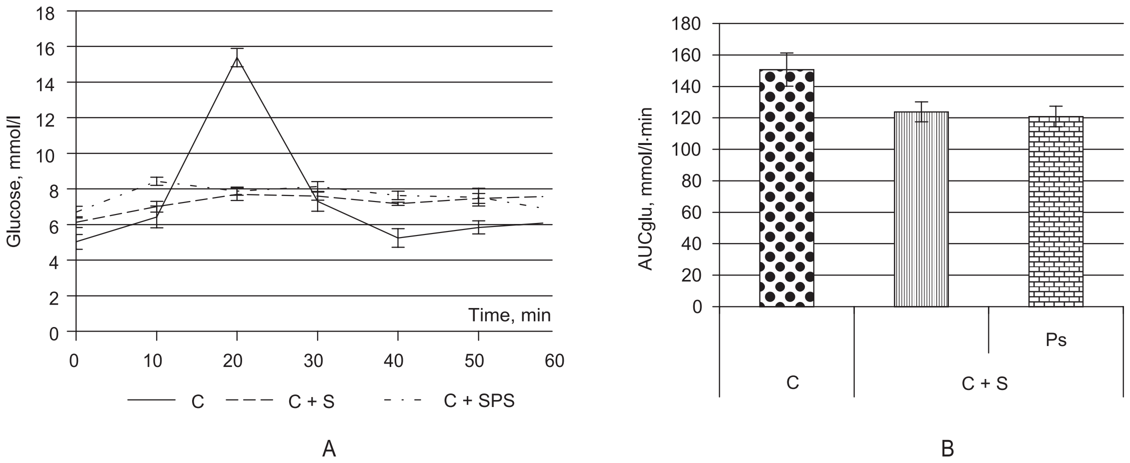

Fig. 7. *A) Effect of yacons root tubers water suspensions on blood glucose concentration changes of healthy rats. B) The area under the glycemic curves in healthy animals that were treated with the water suspensions of yacons root tubers (M ± m, n = 4-8)*

stabilized and stabilized forms of the suspensions caused a decrease in *AUCglu,* correspondingly, by 17.83 % and 19.75 %, compared with the control (Fig. 7).

The phytochemical study of yacon root tubers has indicated that they contain phenol compounds (caffeic, chlorogenic and ferulic acids), L-tryptophan (an essential amino acid) and fructooligosaccharides (FOS), mostly inulin [7, 11]. The essential amino acid L-tryptophan normalizes tolerance to hydrocarbons, increases the level of insulin and, as a result, decreases the level of hyperglycemia. It is also known that L-tryptophan exerts positive effects on carbohydrate metabolism in hepatocytes due to increased activity of glucokinase, hexokinase and glucose-6-phosphate dehydrogenase, which are the key enzymes of the carbohydrate exchange [16].

The fact that yacon root tubers produce the most significant hypoglycemic effect may be attributed to a high FOS content. Such compounds change the kinetics of macronutrients absorption, especially of carbohydrates. FOS molecules, which are not decomposed in the stomach, absorb a great amount of ingest glucose and, consequently, interfere with its absorption into blood, which promotes a decrease in the level of blood sugar after meals. The stable decrease in the level of glucose causes normalization of self insulin production by pancreatic cells. Furthermore, FOS are hydrolyzed into smaller fragments and free fructose by intestine microflora. Fructose metabolism does not need insulin, which allows to avoid the "energy hunger" of cells and return the metabolism of diabetics to normal. Moreover, short fragments of FOS molecules by inserting into the cell membrane facilitate the transportation of glucose into the cell [14]. FOS also modulates concentrations of such peptides as GIP (glucose-dependent insulinotropic polypeptide) and GLP-1 (glucagon-like peptide 1) which-regulate insulin release after meals [5].

The findings of the research into the hypoglycemic effects of yacon water extracts and suspensions may become useful for the development of functional foods, which will allow to avoid harmful effects of hyperglycemia under DM.

CONCLUSIONS

The comparative analysis of hypoglycemic effects produced by the ground and root parts of yacon, which have been evaluated by the glucose tolerance test and the area under glycemic curves, indicates that the best hypoglycemic effect is brought about by yacon root tubers when they are used in the form of stabilized suspensions.

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ВПЛИВ ЯКОНУ (*SMALLANTHUS SONCHIFOLIUS* POEPP. & ENDL.) НА ПОСТПРАНДІАЛЬНИЙ РІВЕНЬ ГЛЮКОЗИ ЗА УМОВ ЕКСПЕРИМЕНТАЛЬНОГО ЦУКРОВОГО ДІАБЕТУ

Наведені результати досліджень впливу водних екстрактів листя і кореневих бульб якону та суспензій кореневих бульб якону на толерантність до глюкози у тварин з експериментальним цукровим діабетом (ЕЦД). Показано, що досліджувані екстракти та суспензії якону у дозі 500 мг/кг володіють вираженою гіпоглікемічною дією за умов експериментального цукрового діабету. На основі проведених тестів толерантності до глюкози та аналізу площі під глікемічними кривими встановлено, що найкраща гіпоглікемічна дія притаманна кореневим бульбам якону у формі стабілізованої суспензії.

Ключові слова: якон (*Smallanthus sonchifolius* Poepp. & Endl.); цукровий діабет; гіпоглікемічний ефект

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ВЛИЯНИЕ ЯКОНА (*SMALLANTHUS SONCHIFOLIUS* POEPP. & ENDL.) НА ПОСТПРАНДИАЛЬНЫЙ УРОВЕНЬ ГЛЮКОЗЫ В УСЛОВИЯХ ЭКСПЕРИМЕНТАЛЬНОГО САХАРНОГО ДИАБЕТА

Представлены данные о влиянии водных экстрактов листьев и корневых клубней якона, а также суспензий корневых клубней якона на толерантность к глюкозе у животных с экспериментальным сахарным диабетом (ЕЦД). Показано, что исследуемые экстракты и суспензии якона в дозе 500 мг/кг обладают выраженным гипогликемическим действием в условиях экспериментального сахарного диабета. На основе проведенных тестов толерантности к глюкозе и анализа площади под гликемическими кривыми установлено, что более выраженным сахароснижающим действием обладают корневые клубни якона в форме стабилизированной суспензии.

Ключевые слова: якон (*Smallanthus sonchifolius* Poepp. & Endl.); сахарный диабет; гипогликемический эффект

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