Yacon's (Smallanthus Sonchifolius Poepp. and Endl.) Effects on Postprandial Glucose under Experimental Diabetes Mellitus

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INTRODUCTION

Epidemiological and clinical studies revealed that exogenously-induced postprandial hyperglycemia affects the development of diabetes mellitus, as well as, increase the risk of its complications (Charpentier, 2006).

In recent years, functional food intake increased 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, maintaining of health and for prevention of modern civilization diseases such as atherosclerosis, obesity, oncological diseases, osteoporosis and diabetes mellitus (DM) (Pankiv, 2013).

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 micro-organisms (Bifidobacterium and Lactobacillus), antioxidants, vitamins (A, E, C and beta-carotene), minerals (calcium etc), microelements (iron, zinc, fluorine, selenium etc) and flavonoids (phytoestrogens and quercetin etc) (Happarov and Wojtkiewicz, 2002).

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 a polysaccharide of root tubers, which consists of D-fructofuranose residues linked by β-2, 1-glycosidic bonds. The polysaccharide chain ends with a residue of α-D-glucopyranose. As for dietary properties of inulin, they are caused by β-2, 1-glycoside linkage. Since human enzymes are not capable of cleaving the bond, inulin remains intact while going through almost the entire 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 (Yorhacheva et al., 2010).

Yacon (Smallanthus sonchifolius Poepp. and Endl. or Polymnia sonchifolia Poepp. and Endl.), an inulin-containing plant, is a potential source of functional foods. It was recorded in the literature that water extracts of yacon leaves are 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 the study of Pankiv (2013).

Shortly, in the 21st century, the hypoglycemic effect of the...
water extract of yacon leaves was proven by findings on the model of type 1 experimental diabetes mellitus (EDM) (Aybar and Sánchez, 2001; Honoré and Genta, 2015).

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 was carried out on Wistar rats with the weight of 130 to 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 + E70) and 0.5 g / kg /day (C + E500);
3) Control animals that were treated orally with water extract of yacon root tubers at doses 0.07 g / kg /day (C + E70) and 0.5 g / kg /day (C + E500);
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 + S59);
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 + E70) and 0.5 g / kg /day (DM + E500);
7) Animals with diabetes that were treated orally with yacon root tubers water extract at doses 0.7 g / kg /day (DM + E70) and 0.5 g / kg /day (DM + E500);
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 + S59).

Induction of diabetes

Type 1 EDM was induced by an intra-abdominal 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 h). 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 100ºC and 45 min at 20ºC. The extracts were filtered and vaporized in the vacuum to receive dry remnant by LABOROTA 4001 rotor vaporizer (Heidolph, Germany) at 60 to 65ºC. 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 h 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 affected the level of blood sugar (the null point is on an empty stomach, in 10, 20, 30, 40, 50 and 60 min respectively after glucose taking). The calculated integral index of the area under the glycemic curve (AUCgly), 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 (Yeh, 2002).

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≤0.05.

RESULTS AND DISCUSSION

Previously performed screenings 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 (Horbunilska et al., 2014). Considering THE obtained results, we decided to conduct a study of influence of extracts with the 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 (Pankiv, 2013).

It was revealed that glycemic curves were considerably changed in animals with EDM. It established a dramatic increase in glucose concentrations after glucose loading in their blood on the 10th minute and a further increase on the 20th minute of the experiment. As for control animals, the maximal glucose concentration was on the 20th minute and a full return to the initial level of glucose was noted on the 30th minute of the experiment, whereas under EDM glucose concentrations were back to normal on the 50th minute (Figure 1A).

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 10th minute followed by its dramatic decrease to the initial values. 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 (Figure 2A). It also caused a decrease in AUCglu as compared with EDM, by 44.11%. On the other hand, the dose of 0.7 g/kg did not produce such an effect (Figure 2B). 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 gastro-intestinal tract by inhibiting sodium-glucose transporter-1 (S-GLUT-1). It is known that chlorogenic acid inhibits glucose-6-phosphatase, thus, affecting the metabolism of hydrocarbons (glycolysis, glycogenolysis and gluconeogenesis) (Honore and Genta, 2012). 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 (Baroni and Suzuki-Kemmelmeyer, 2008; Honore and Genta, 2015; Valentová and Ulrichová, 2003). The synergistic action of all the compounds leads to the marked hypoglycemic effect.

The glycemic peak decreased on the 20th minute due to
Figure 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). *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.

Figure 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). *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.

the introduction of yacon leaves water extract in the examined concentrations to control animals (Figure 3A). Despite changes in glycemic curves, AUCglu index remained the same as control values (Figure 3B). 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 that an increase in glucose concentration on the 20th minute after the hydrocarbon loading was followed by its gradual decrease on the 30th minute. The level of glucose reached its initial value on the 50th minute of the experiment (Figure 4A). 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% as compared with the analogous index in animals with EDM (Figure 4B).

While researching into the hypoglycemic effect of the yacon root tubers extract in the examined concentrations, it
was revealed that it produced 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 30th minute. In the dose of 0.5 g/kg, the peak was less marked (20th minute) and the repeated increase in glucose concentration occurred on the 40th minute after the hydrocarbon loading (Figure 5A). The shift and splitting of the peak may be attributed to inhibition or slowing-down of glucose assimilation in the gastro-intestinal tract under the action of biologically active substances of yacon root tubers. A decrease in AUC{sub}glu (by 32.74%, compared with control) when treated with the dose of 0.7 g/kg also testified the changes in the hydrocarbon assimilation (Figure 5B).
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, non-explosive 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, micro-organisms 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 (Chueshov and Hladuh, 2010). Therefore, we have chosen to administer the plant in the form of suspension. 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 (Georgiyevsky and Konev, 1996).

The study on glucose loading 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 10th and 20th minutes with further decrease on the 30th minute and return to the initial level already on the 40th minute of the experiment. On the other hand, a stabilized suspension with glucose loading led to a slight increase in glucose concentrations on the 10th minute. On the 20th to 50th minutes, the glucose level was within the basal value. It should be noted that on the 60th minute 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% respectively, as a result of changes in the glycemic curves following administration of the non-stabilized and stabilized suspensions (Figure 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-stabilized and stabilized forms of the suspensions caused a decrease in AUCglu, correspondingly, by 17.83 and 19.75% as compared with the control (Figure 7).

The phytochemical study of yacon root tubers indicated that they contain phenol compounds (caffeic, chlorogenic and ferulic acids), L-tryptophan (an essential amino acid) and fructooligosaccharides (FOS), mostly inulin (Grethel et al., 2012; Lobo et al., 2014). 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 (Yan and Suzuki, 1999).

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 is hydrolyzed into smaller fragments and free fructose by intestine microflora. Fructose metabolism does not need insulin, which allows avoiding the “energy hunger” of cells and returning 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 (Roberfroid, 2007). FOS also modulates concentrations of such peptides as GIP (glucose-dependent insulinitropic polypeptide) and GLP-1 (glucagon-like peptide 1) which regulate insulin release after meals (Delzenne and Kok, 1999).

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 avoiding harmful effects of hyperglycemia under DM.

Conclusions

The comparative analysis of hypoglycemic effects produced by the ground and root parts of yacon which was 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.

REFERENCES


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