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Sugar-lowering effects of Galega officinalis L.

Działanie hipoglikemizujące *Galega officinalis* L.

INTRODUCTION

Diabetes mellitus (DM) is one of the most common diseases, because of the growing number of patients in all countries (according to WHO there are more than 150 million patients in the world today) [11]. The social problem of diabetes is in early disability and mortality which is caused by the presence of late complications – micro and makroangiopathia. Along with traditional methods of DM treatment nowadays there are more and more widely used alternative tools that help facilitate the condition of patients. These include herbal medicine, which is used as an independent method in easy flowing DM, has a subsidiary character with average disease severity and is used as a diagnostic tool with a heavy course of diabetes and its complications [1, 2, 8, 9]. *Galega officinalis* L. is a perennial grass plant from the legume family (*Fabaceae*), which is used in alternative medicine to treat DM. In Bulgaria, the UK and the USA it is the official plant, which is prescribed in mild diabetes form. Although the hypoglycemic effects of *Galega officinalis* were discovered back in 1927, the recorded data on hypoglycemic properties of herbs and seeds of this species is controversial. [3, 7, 10, 11].

The aim of the work is to study hypoglycemic properties of agents obtained from the alcohol extracts of *Galega officinalis* on the model of experimental diabetes.

MATERIAL AND METHODS

The experiments were performed on white breedless male rats weighing 110–150 g, kept in standard vivarium conditions. Experimental diabetes was caused by inter-abdominal injection of streptozotocin (Sigma, USA) in a dose of 7 mg per 100 g body weight of animals. Animals in whose blood glucose concentration was 8mmol/l or higher (after 18-hour hunger) were used for research.

Blood for analysis was taken from the tail vein on the 3rd, 6th, 9th and 12th days of medicine injection, and also on the 7th day after injection of investigational medicine cessation. Glucose

concentration was determined with the help of glucoseoxidaze method via "Diahlyuk" set (Institute of Cell Biology NAS of Ukraine, Lviv).

Galega officinalis, introduced in the Botanical Garden of Ivan Franco Lviv National University, was used for the studies. The ground part was collected during flowering, then it was dried out and alcohol extract was produced by infusion in 96% ethanol for 12 hours at a ratio of 1:5 at room temperature. Under the condition of ethanol extraction glycosides, flavonoids, saponins and partly alkaloids move to the extract.

The resulting *Galega officinalis* alcoholic extract was divided into water (alkaloid-containing) and chloroform fractions. Alkaloids are found in plants in the form of bases or salts with certain organic acids. Alkaloids salts are soluble in water and alcohol (methyl, ethyl) and insoluble in ether and hydrocarbons. Therefore, for extraction alkaloids in the form of salt water and alcohol are often used. Under these conditions the so-called extractive substances (proteins, resin, tannins, mucus, etc.) come into solution. To get rid of these ballast substances, the method of previous raw materials purification by processing hydrochloric acid and chloroform is used. During this process free alkaloids are converted into salts, which are not soluble in chloroform, and solvent extracts only neutral and acidic extractive substances.

Considering the above, to obtain medications with certain composition and for achieving a certain degree of standardization, alcohol extract was evaporated in vacuum using rotary evaporators LABOROTA 400 (Heidolph, Germany) at the temperature of +50 ... +55 °C to obtain dense residue extract of jam consistency. A few drops of 0.1 N hydrochloric acid (to pH = 2), 9 ml H_2O (to obtain a homogeneous mass) and equal volume of chloroform were added to 14 g of maximum evaporated original alcohol extract. After shaking, the samples were centrifuged for 10 min at 1500 rpm. The obtained fractions, 1 – water (of dark brown color), 2 – chloroform (light-green colored) were evaporated in vacuum at the temperature of +50 to +55 °C to obtain solid residue. These evaporated remains from the original water extract and its fractions were dissolved in water. Water solution of the original alcohol extract was called medicine # 1, and solution of its aqueous fractions – medicine #2.

Animals were divided into five groups: first – control animals, the second – streptozotocin-treated animals; third – healthy animals, in which medicine # 1 for 12 days was injected per os (0.6 g / kg body weight in 1 ml volume), fourth – streptozotocin-treated animals, in which medicine # 1 for 12 days was injected per os (0.6 g / kg body weight in 1 ml volume), fifth – streptozotocin-treated animals which medicine # 2 for 12 days was injected per os in the above mentioned dose.

RESULTS AND DISCUSSION

Our previous studies showed expressed hypoglycemic effect of alcohol extracts of *Galega officinalis* herbs in healthy rats at the concentrations of 2.5, 1.25 and 0.6 g / kg of body weight [4]. Considering these data, we conducted a comparative study of glucose concentration dynamic changes in peripheral blood under the conditions of *Galega* extract introduction in a dose of 0.6 g / kg of body weight to healthy animals and to a model of streptozotocin diabetes.

Under conditions of diabetes, regardless of etiological factors, carbohydrate, protein, fat and mineral metabolism is disturbed. Hyperglycaemia, the main index which inhibited diagnosis, not only provokes excessive formation of oxygen free radicals, but also reduces the activity of protective

mechanisms due to the process of antioxidant enzymes nonenzymatic glycosylation. Protein glycosylation (nonenzymatic accession of glucose to protein amino groups) is one of the important mechanisms in the formation of diabetes vascular complications [2]. Therefore, the most important task in the struggle with complications of diabetes mellitus is to reduce hyperglycemia.

According to literature data, *Galega officinalis* infusions and decoction are used in *phytotherapy* of diabetes. Stable hypoglycemic effect is achieved only in case of prolonged and regular use [5, 6].

During the research, it was established that medicine#1 reduces the glucose level in healthy rats' blood by 39% and 52% on the 3rd and 6th days respectively (Fig. 1).

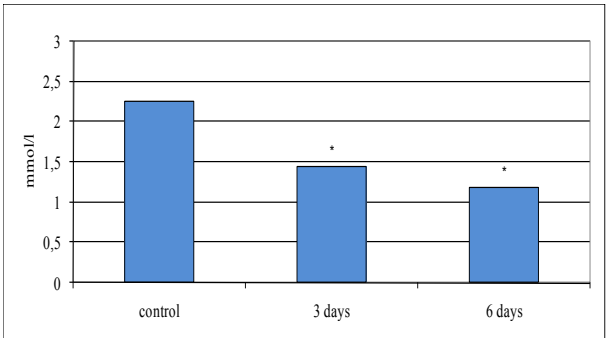

Fig. 1. The dynamics of changes in glucose concentration in blood of healthy rats after administration of medicine #1

Under conditions of medicines from *Galega officinalis* extracts injection on the model of streptozotocin diabetes experimentally the pronounced hypoglycemic action within 12 days of the experiment was confirmed (Fig. 2, 3). The decrease in glucose concentration while entering *Galega officinalis* original alcohol extract in a dose of 0.6 g / kg body weight regarding diabetes was estimated as follows: 51% (3-day), 60% (6-day), 59% (9-day) and 69% (12-day). The most distinct sugar-lowering effect was shown at the terminal stage of the experiment (day 12) at the same time approximation of the glucose concentration to the values inherent in the control animals was noted (Fig. 2).

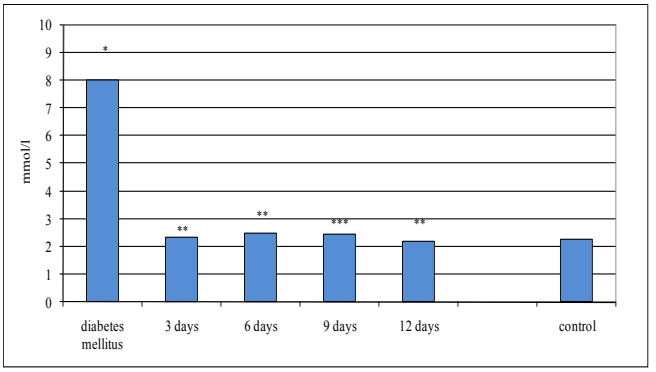

Fig. 2. The dynamics of changes in glucose concentration in blood of rats after administration of medicine #1 under streptozotocin-induced diabetes

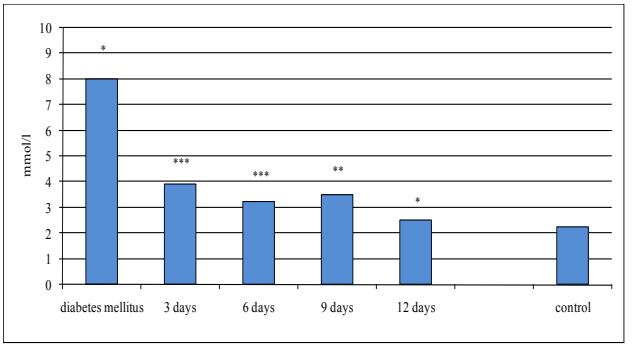

Fig. 3. The dynamics of changes in glucose concentration in blood of rats after administration of medicine #2 under streptozotocin-induced diabetes

The next stage of our work was to compare the performance of the original alcohol extract and its alkaloid-containing fraction. During the injection of medicine #2 a similar pattern was observed – reduction of glucose concentration in animals under the condition of diabetes during 12-days-long experiment (Fig. 3). The target rate was reduced to 71% (3 day) 69% (6 day), 70% (9 day) and 72% (12 day). It should be noted that during the entire period of injection of the drug from this fraction glucose concentration decreased to normal level values.

After the termination of medicine #1 and #2 injection to streptozotocin-treated animals (after 7 days) the glucose level remained lower (by 57% and 37%, respectively) compared with that in diabetic animals to which they had not been injected (Fig. 4). These data indicate a prolonged action of *Galega officinalis* medicines.

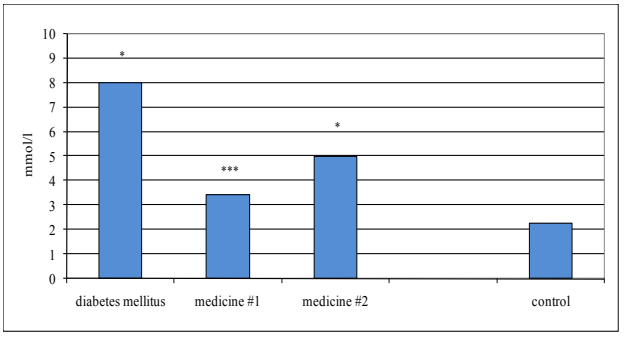

Fig. 4. Glucose concentration in blood of rats on the 7th day after ceasing the administration of *Galega officinalis* medicine under streptozotocin-induced diabetes

It is considered that the *Galega officinalis* active hypoglycemic component is acting within nonpancreas mechanism, increasing glycogen content in the liver and suppressing insulinase enzyme activity [6, 11]. There are reports that long *Galega officinalis* reception restores the activity of β-cells of islets of Langerhans [8].

CONCLUSIONS

The studies confirm the sugar-lowering effect of medicine#1 obtained from an original alcohol extract, in both control and streptozotocin-treated animals. Medicine#2 obtained from the water fraction of total alcohol extract showed a satisfactory sugar-lowering effect under the condition of diabetes as well, but it was less successful in prolonged effects after the termination of the drug injection. The obtained results allow to recommend the usage of medicine from *Galega officinalis* alcohol extract to reduce hyperglycemia in diabetes.

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SUMMARY

The research was carried out to investigate the effects of *Galega officinalis* on the dynamics of glucose concentration changes in the blood of healthy rats and under experimental streptozotocin-induced diabetes. The hypoglycemic effect of the extract both in the control group and in animals with diabetes was shown. It was also found that the medication obtained from ethanolic extract of *Galega officinalis* had a prolonged hypoglycemic effect after ceasing the treatment.

Keywords: *Galega officinalis*, diabetes mellitus, hypoglycemic effect

STRESZCZENIE

Przeprowadzono badania celem oceny wpływu *Galega officinalis* na dynamikę zmian stężeń glukozy we krwi szczurów zdrowych oraz z cukrzycą doświadczalną indukowaną streptozotocyną. Stwierdzono występowanie efektu hipoglikemizującego w obydwu grupach zwierząt. Zaobserwowano również przedłużony efekt hipoglikemizujący, utrzymujący się po przerwaniu leczenia, w przypadku stosowania ekstraktu etanolowego *Galega officinalis*.

Słowa kluczowe: *Galega officinalis*, cukrzyca, działanie hipoglikemizujące