



EFFECT OF TEMPERATURE OF DRYING AND CONCENTRATION OF ETHYL ALCOHOL ON HYPOGLYCEMIC EFFECT OF DRY EXTRACT OF THE BILBERRY OF THE GENERAL

Kh.I. Kurylo., Klishch I.M., Chernyashova V.V., Volska A.S and , Stetsyuk I. O.

I.Horbachevsky Ternopil State Medical University, Ternopil, Ukraine

ARTICLE INFO

Article History:

Received 20th August, 2017

Received in revised form 15th

September, 2017

Accepted 27th October, 2017

Published online 28th November, 2017

Key words:

Diabetes Mellitus, Dry Bilberry Extract, Hypoglycemic Effect, Oral Glucose Tolerance Test

ABSTRACT

Introduction: the study of the effect of drying temperature and the concentration of ethyl alcohol on the hypoglycemic effect of dry bilberry extract on the model of oral glucose tolerance test on rats.

Aim of The Study: The purpose of this study was to study the effect of drying temperature and the concentration of ethyl alcohol on the hypoglycemic effect of dry bilberry extract on the model of oral glucose tolerant test on rats. Oral Glucose Tolerance Test (OTTG) is a method of studying neurohormonal regulation of glycemia due to its change in the loading process.

Materials and Methods: The object of research is dry bilberry extract (*Vaccinium myrtillus* L.). Experiments were performed on white non-linear male rats weighing 175 ± 20 g with normal carbohydrate homeostasis, which was assessed by basal glycemia and glucose tolerance in the model of oral glucose tolerance test. The intact control group received-distilled water. The animal is randomly assigned to the groups (n = 6): 1-intact animals, control; 2-animals of control pathology receiving an intragastric 40% glucose solution at a rate of 3 g / kg body weight; 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13-animals fed a dry bilberry extract at a dose of 50 mg / kg at different drying temperatures (500, 700, 900, 1100, 1300, 1400) pre-dried.

At the next stage of the study, the hypoglycemic effect of blueberries was estimated at a variable concentration of ethyl alcohol. Experiments were performed on white nonlinear male rats weighing 160 ± 20 g, with normal carbohydrate homeostasis, which was assessed by basal glycemia and glucose tolerance in the model of oral glucose tolerance test. The group of intact animals under the same scheme received-distilled water. The animal is randomly assigned to the groups (n = 6): 1-intact animals, control; 2-animals of control pathology receiving an intragastric 40% glucose solution at a rate of 3 g / kg body weight; 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13-animals that were injected with dry extracts of the goat and blueberries in a dose of 50 mg / kg with a different content of ethyl alcohol (0%, 10%, 20 %, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 96% respectively) of pre-dried.

Results: So, the most pronounced hypoglycemic effect of dry bilberry extracts of the usual dose of 50 mg / kg, which was extracted with 60 % ethyl alcohol and manifested at a drying temperature of 50 ° C.

Conclusion: Intragastric administration of glucose at a dose of 3 g / kg led to an increase in glucose levels at 30, 60, 90, 120, 180 min in all groups of animals, compared with the control group of animals. It was found that dry bilberry extract in a dose of 50 mg / kg dried at a drying temperature of 50 ° C and the most optimal concentration of alcoholic alcohol-60 % for extract of bilberry extracted, showed a higher hypoglycemic activity compared with other experimental groups. The obtained data testify to expediency of further experimental researches with the purpose of creation of the antidiabetic preparation.

Copyright © Kh.I. Kurylo. *et al* 2017, This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original work is properly cited.

INTRODUCTION

Increasingly urgent issue, to create drugs plant-based. Today, medicinal plants and medicines are widely used in medicine and are in high demand by the population of not only Ukraine but also the world, the share of which in different countries makes 30-50 % of the total volume of medicinal products. A distinctive feature of many herbal preparations is a complex and complementary effect of a combination of biologically active substances that they contain in their composition.

Therefore, the main task of modern pharmacology is the study of medicinal plant material (RLC) and the creation on its basis of effective medicines. One of the most

valuable sources of LRS is blueberries (*Vaccinium myrtillus* L.), fruits, shoots and leaves, which are widely used in folk and officinal medicine in the form of decoctions. Useful properties of common bilberry leaves and shoots are most often used in diabetes mellitus (DM) as a hypoglycemic agent. In mild forms of the disease, it is used as one of the main drugs, and in more severe stages, it is used in combination treatment with oral hypoglycemic agents [1].

Hypoglycemic activity is due to the presence of simple phenols in the raw material (arbutin); polyphenols, namely chlorogenic acid, which inhibits glucose-6-phosphatase, which catalyzes the final stage of glycogenolysis and

*Corresponding author: Kh.I. Kurylo

I.Horbachevsky Ternopil State Medical University, Ternopil, Ukraine

gluconeogenesis; and flavonoids that improve microcirculation of tissues due to membrane-stabilizing and antioxidant action and improve the metabolism of insulin-dependent processes. According to phytochemical studies, blueberries contain glycosides of myrtiline and neomyritin (the so-called "plant insulin") [2]. The beneficial effect of blueberries on oxidative phosphorylation processes in liver mitochondria has also been established [3].

With the prolonged use of synthetic hypoglycemic agents, marked development of resistance to them, marked deterioration of lipid metabolism, accelerated development of atherosclerosis of large and small vessels, the formation of so-called diabetic retinopathy, nephropathy, angiopathy of the extremities.

Given the wide range of lesions and complications of diabetes by various organs and systems, in addition to reducing blood glucose, there are numerous indications for the administration of different mechanisms of action of medicinal plants. Possible pathogenetic mechanisms of their effect include: influence on immune processes, inhibition of absorption of glucose in the gastrointestinal tract, stimulation of regeneration of β -cells of Langerhans islands, normalization of lipid peroxidation, increased activity of sorbitol dehydrogenase and glycogenic stimulation. The plant materials can act as an auxiliary resource of microelements, vitamins and, accordingly, play a role in ensuring the enzymatic reactions of aerobic oxidation of glucose, both at the stage of glucose splitting to pyruvic acid, and at the stage of oxidative decarboxylation of pyruvic acid. Diabetes mellitus is a global medical and social problem that is accompanied by the development of secondary chronic complications that lead to early disability and high mortality.

According to the International Diabetic Federation (IDF), today, about 382 million people in the world suffer from diabetes. In 2035, this figure will increase by 55 %, mainly due to patients with type 2 diabetes [4]. Type 2 diabetes is a disease that progresses over time, but the rational and systematic use of antidiabetic synthetic drugs and phytopreparations can significantly delay the onset of insulin therapy.

The purpose of this study was to study the effect of drying temperature and the concentration of ethyl alcohol on the hypoglycemic effect of dry bilberry extract on the model of oral glucose tolerant test on rats.

MATERIALS AND METHODS

The object of our research is the overground part of the medicinal plant material-the leaves of blueberries of the usual (*Vaccinium myrtillus* L.). For research leaves were chosen for the study, because they exceed the shoots by 1.5-2 times the content of flavonoids, which, according to the literature, are responsible for the sugar-lowering activity [5] from which the dry extract was obtained. As a result of our studies, the hypoglycemic effect of dry extract of blueberries was established and its effective dose of 50 mg / kg was established.

The first step in our study was to determine the effect of drying temperature on the hypoglycemic effect.

Experiments were performed on white non-linear male rats weighing 175 ± 20 g with normal carbohydrate homeostasis, which was assessed by basal glycemia and glucose tolerance in the model of oral glucose tolerance test. A group of animals of intact control (IC) received a solvent-distilled water. The animal was randomized to the groups ($n = 6$) by the blind sample: 1 group-intact animals, control (IC); 2 group-animals of control pathology receiving an intragastric 40% glucose solution at a rate of 3 g / kg body weight; 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13-animals fed a dry bilberry extract at a dose of 50 mg / kg at different drying temperatures (50° C, 70° C, 90° C, 110° C, 130° C, 140° C) pre-dried.

At the next stage of the study, we evaluated the hypoglycemic effect of blueberries with the variable alcohol concentration of ethyl alcohol. Experiments were performed on white nonlinear male rats weighing 160 ± 20 g, with normal carbohydrate homeostasis, which was assessed by basal glycemia and glucose tolerance in the model of oral glucose tolerance test. A group of animals of intact control (IC) under the same scheme received a solvent-distilled water.

The animal was randomized to the groups ($n = 6$) by the blind sample: 1 group-intact animals, control (IC); 2 group-animals of control pathology receiving an intragastric 40% glucose solution at a rate of 3 g / kg body weight; 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13-animals that were injected with dry extracts of the goat and blueberries in a dose of 50 mg / kg with a different content of ethyl alcohol (0%, 10%, 20 %, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 96% respectively) of pre-dried.

The glucose tolerance test was performed after at least six hours of starvation of animals to determine the level of glycemia. The hypoglycemic effect of dry extract of blueberries was evaluated on the ability to reduce glucose levels at the maximum of hyperglycemia after glucose loading by plotting a graph of a glycemic curve (on an empty stomach-0 point, 30, 60, 90, 120, 180 min after glucose loading). Blood glucose concentration was determined using test strips, "Accu-Chek performa" glucose meter. Blood for research was obtained from the tail vein of the rats by distal resection of the tail.

All manipulations were conducted in compliance with the principles of bioethics in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes (European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes, Strasbourg, 1986), the Law of Ukraine "On the Protection of Animals from Cruel Treatment" (№ 1759-VI of 15.12.2009) and EU Directive 2010/10/63 on animal experiments.

Statistical processing of the obtained results was performed using ANOVA-test using nonparametric criteria using Statistica v.10.1 program.

RESULTS

Oral glucose tolerance tests, in addition to diagnosis of diabetes, are also prominent in assessing the effectiveness

of hypoglycemic drugs, and can also be used to find new agents with hypoglycemic properties.

Glucose-a powerful regulator of the activity of β - cells of the islet apparatus of the pancreas. An increase in the concentration of glucose in the blood stimulates the release of insulin in the blood, indicating the balance between the rate of glucose supply to the extracellular fluid and its utilization.

In the process of assessing the hypoglycemic effect of medicinal plants, the study of the effectiveness of assimilation of exogenous glucose is used as an additional indicator of changes in carbohydrate metabolism. Oral Glucose Tolerance Test (OTTG) is a method of studying neurohormonal regulation of glycemia by its change in the process of loading.

The first increase in glucose concentration at the 30th minute of the experiment after the load reflects the reflex stimulation of sympathetic nerves, which occurs when glucose enters the digestive canal.

At the 60th minute after the introduction of carbohydrate per os the maximum increase in glucose concentration in the blood is observed, which is related to the rate of absorption of carbohydrates (as determined by the state of the intestine wall) and the functioning of the liver.

Decrease of glucose level for 90 minutes and gradual approximation to the initial level occurred up to 120 minutes, within which it was observed until the final terms of the experiment (180 minutes).

The absence of changes in the nature of the curves within 0-30 minutes after the carbohydrate loading in the application of a dry extract of blueberries common, indicates the absence of exposure of active substances to the strength of reflex stimulation of sympathetic nerves, which occurs when glucose enters the digestive canal. Reducing the severity of the peak on the glycemic curve when applied to the substances studied is an effect on the rate of absorption of carbohydrates, and less pronounced hypoglycemic phase is the effect of the investigated extracts on the production of insulin.

The lower branch of the curve reflects the production of insulin and depends on the functional state of the parasympathetic nervous system and functioning of the pancreas and is a hypoglycemic phase. The last point on the glycemic curve reflects the state of the glucose utilization system, and is normally equal to or less than 10-15 % of the initial glucose level.

As a result of the primary pharmacological screening of hypoglycemic activity of blueberries, it was established and confirmed its ability to reduce the level of glucose in the blood, which had a dose-dependent nature. The hypoglycemic activity of the dry extract in a dose of 50 mg / kg was the most clear and pronounced, which was used in our further studies.

The first step in our study was to determine the effect of drying temperature on the hypoglycemic effect. Preliminary administration of animals to a dry bilberry extract at a dose of 50 mg / kg at different t drying conditions (50 ° C, 70 ° C, 90 ° C, 110 ° C, 130 ° C, 140 ° C) causes a significant reduction in glucose levels in

rat blood through 30, 60, 90, 120, 180 min. after sugar loading in all groups of animals (Fig. 1).

During the test, it was found that the maximum increase in glucose concentration in the blood of animals of the CP was observed after 30 minutes after glucose loading (Fig. 1). As seen from the presented glycemic curve, the glycemic index for 60 minutes of experiment almost 1.5 times exceeded the indicator in the group of animals IR and remained at a relatively high level for the next 30 minutes, and then gradually decreased. Three hours after the injection, the concentration of glucose in the blood of animals of the CP remained higher relative to the group of IC.

As can be seen from the data presented in Fig. 1 dry extract of ordinary blueberries (*V. myrtillus*) at a drying temperature of 90 ° C, 110 ° C, 130 ° C, 140 ° C did not have a noticeable effect on the dynamics of changes in glucose concentration during the test. Glycemic curves reflecting the effects of extracts at different drying temperatures did not differ significantly from the glycemic curve of the CP: increased glucose concentration after 30 minutes after glucose loading, maintaining a relatively high level for the next 30 minutes, and a subsequent gradual decrease in the level of glycemia. In the group of animals receiving the extract of blueberries of the usual (*V. myrtillus*), which was dried at 50 ° C, there was a progressive increase in glucose concentration; the maximum values of the glycemic index reached 60 minutes from the moment of glucose loading and remained at a significantly higher level compared with the control group until the end of the experiment and showed significant hypoglycemic effect in the glucose tolerance test.

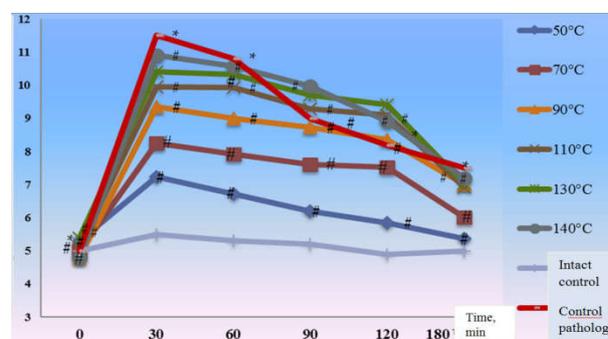


Fig 1 Influence of temperature of drying leaves of bilberries on its hypoglycemic effect

Note. *-p <0,01-statistically significant differences in comparison with indicators of intact control;
#-p <0,01-statistically significant differences in comparison with the indicators of control pathology;

A positive correlation between the high temperature and the hypoglycemic effect in animals ($r = 0.90$) was established (Fig. 2). This fact indicates that when the temperature rises, the healing properties of the medicinal raw blueberries are usually lost (the average value at 50 ° C is close to the average value of the intact) (see Figure 2).

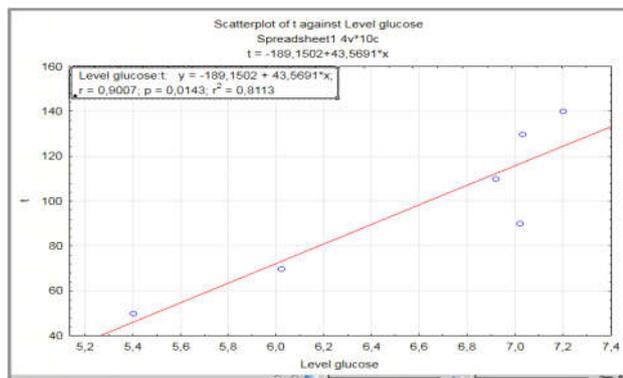


Fig 2 Correlation between drying temperature and hypoglycemic effect in animals of control group and group with dry extract of blueberries common

Consequently, the optimum temperature for drying bilberry leaves for a better hypoglycemic effect of 50 °C. At the next stage of the study, we evaluated the hypoglycemic effect of blueberries with the variable alcohol concentration of ethyl alcohol. The evaluation of the glyceimic curve under the action of a dry blueberry extract showed that the curve, which was the most similar in appearance, was based on the results of the effect on the level of glucose dry extract prepared on 60 % ethyl alcohol. Having fully appreciated the results obtained, it was concluded that for further research, dry extract of blueberries of the usual prepared on 60 % ethyl alcohol should be used. It was observed that at the end of the test, the level of glycemia was not significantly different from the initial values in the rat IC.

In animals that received an extract of 50 mg / kg at 60 % alcohol, the ethyl hypoglycemic effect was moderate and mild but had a significant difference from the control group ($p < 0.01$) and showed a higher hypoglycemic activity compared to other experimental groups Further increase of the concentration of ethyl alcohol did not lead to a statistically increased hypoglycemic effect (see Figure 3).

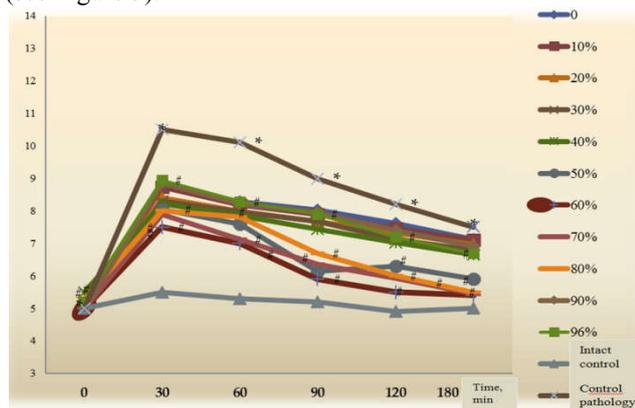


Fig 3 Choosing the optimal concentration of extractant for better hypoglycemic action of blueberries common

Note. *- $p < 0,01$ -statistically significant differences in comparison with indicators of intact control;
&- $p < 0,01$ -statistically significant differences in comparison with the indicators of control pathology;

A dry bilberry extract of 50 mg / kg, extracted with 60 % ethyl alcohol, showed a higher hypoglycemic activity than other experimental groups. Further increasing of the

concentration of ethyl alcohol did not lead to a statistically increased hypoglycemic effect (see Fig. 3).

For the first time, there were significant significant ($r = 0,50-0,69$) direct correlation relationships between the control animals and groups of dry bilberry extracts (Fig. 4).

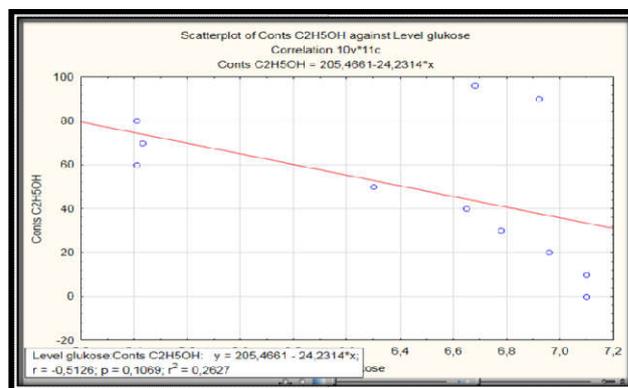


Fig 4 Correlation between index % of alcohol and hypoglycemic effect in animals of control group and group with dry extract of blueberries of ordinary

Through the experiment, direct dependence of the concentration of ethyl alcohol on the hypoglycemic effect of the investigated extracts was established. This ratio is necessary and sufficient for obtaining a remedy with hypoglycemic effect. The given ratio is optimal in the technological terms in terms of obtaining extracts in industrial conditions and the rational use of alcohol.

Consequently, the most optimal concentration of ethyl alcohol for blueberries is usually 60 %, which was taken into account in our further research.

CONCLUSION

Taking into account the pace of prevalence, multiple morbidity of injuries, early disability and high mortality, diabetes is one of the most serious problems in the modern world. A significant disadvantage of the groups of hypoglycemic agents used today is a number of side effects. For modern medicine, medicinal plants and phytopreparations on their basis are the main source for the search and development of new approaches and strategies for the pharmacotherapy of diabetes mellitus.

The analysis of the data obtained allows us to conclude that intragastric administration of glucose at a dose of 3 g / kg resulted in an increase in glucose levels in 30, 60, 90, 120, 180 min in all groups of animals, compared with the control group of animals.

It was found that dry bilberry extract in the dose of 50 mg / kg dried at a drying temperature of 50 °C, and the most optimal concentration of ethyl alcohol for the extracted bilberry extract was shown at 60 % ethyl alcohol, which showed a higher hypoglycemic activity compared with other experimental groups for a better hypoglycemic effect.

Despite the fact that bilberry extract is one of the most popular phytoprotheses among patients with diabetes in the population of Ukraine, the mechanisms of its therapeutic effect have not been established to the end, and therefore further research is advisable to focus on

studying the hypoglycemic properties of this plant extract. In our opinion, the active phytocomponents of blueberries can become a promising raw material for the search and creation of new effective agents with hypoglycemic properties. The obtained data testify to expediency of further experimental researches with the purpose of creation of the antidiabetic preparation.

References

- Bioactivity of Flavonoids on Insulin-Secreting Cells / M. Pinent, A. Castell, I. Baiges [et al.]. // Comprehensive Reviews in Food Science and Food Safety.-2008.-Vol. 7, № 4.-P. 299-308.
- Complete assignment of Bilberry (*Vaccinium myrtillus* L.) anto-cyanins separated by capillary zone electrophoresis / T. Ichiyanagi, Y. Kashiwada, Y. Ikeshiro [et al.]. // *Chem. Pharm. Bull.* -2004.-№ 2.-P. 226-229.
- Martineau L. C. Anti-diabetic properties of the Canadian low-bush blueberry *Vaccinium angustifolium* / [L. C. Martineau, A. Couture, D. Spoor et al.] // *Ait. Phytomedicine.*-2006.-Vol. 13.-P. 612-23.
- International Diabetes Federation. IDF Diabetes Atlas.- 6th edition.-2013.-P. 7-9.
- Malviya N. Antidiabetic potential of medicinal plants / N. Malviya, S. Jain, S. Malviya // *Acta. Pol. Pharm.*-2010.-Mar-Apr.-Vol. 67, № 2.-P. 113-118.
