



EFFECT OF HYPOGLYCEMIC COLLECTION ON ENZYME ACTIVITY AND INTENSITY OF GLUCONEOGENESIS UNDER CONDITIONS OF EXPERIMENTAL DIABETES.

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The activity of tissue enzymes - hexokinase and phosphorylase in the liver and muscle tissue and the effect of collection on the rate of formation of glucose from its precursors under conditions of hyperglycemia of alloxan origin in the liver were studied. The results of the study of the hypoglycemic properties of the herbal preparation are presented. The main links of glucose metabolism in the liver and muscles under conditions of experimental diabetes under the influence of the collection were studied with a simultaneous comparison with the effect of insulin on these processes.

Keywords: antidiabetes, toxicity, insulin, gluconeogenesis, experimental, intact, hyperglycemia, adenylate cyclase, glucose diabetes mellitus, alloxan mellitus, phosphorylase, hexokinase, enzyme, alloxan hydrate, decapitation, Fiske-Subbaru, liver, skeletal muscle, glycolysis.

Diabetes mellitus is a consequence of a violation of insulin regulation of a number of cells in the body. Diabetes is a common disease and is the third leading cause of death after cardiovascular disease and cancer. Type II diabetes occurs in adulthood, usually after the age of 40. It develops gradually, the symptoms are moderate, acute complications are rare. Due to its high prevalence, early disability and reduced life expectancy of patients, diabetes mellitus is one of the most important medical and social problems. The study of the mechanisms of insulin regulation, the etiology and pathogenesis of diabetes mellitus, the search for new methods of treatment are carried out in the world very widely and intensively. Recently, the main tasks of research are the transition from the diagnosis of diabetes to its prediction, from treatment to prevention. The problem of finding medicinal plants with a hypoglycemic effect that normalizes metabolic processes in diabetes mellitus for practical medicine remains relevant [1].

Pharmacotherapy in diabetes mellitus includes aspects of enhancing insulin secretion depending on the type of diabetes, replacing insulin in case of its deficiency and normalizing existing metabolic disorders. Synthetic derivatives of sulfonylurea and biguanides and their subsequent analogues remain the main oral treatment for patients with non-insulin-dependent diabetes..

Herbal preparations are of particular interest. In folk medicine, it is known that Avicenna used raw materials and the collection of local plants to compensate for diabetic conditions.

However, which substance or group of substances is the active principle is not known and practically unexplored. In the chemistry of herbal preparations, there is no information about its carbohydrate composition. In this regard, the isolation and study of the hypoglycemic active start of the collection, which consists of two local plants

(*Morus alba*, *Plantago major* (leaves of mulberry and plantain)) in order to create a pharmacological agent on its basis, it seems very interesting. [2].

Gluconeogenesis - the synthesis of glucose from non-carbohydrate precursors, mainly from amino acids and metabolites of intermediate metabolism - is a specific function of hepatocytes and cells of the cortical layer of the kidneys. The most important function of gluconeogenesis is to maintain blood glucose levels in the face of reduced food intake and glycogen stores.

This paper presents the results of studying the effect of collection on the activity of hexokinase and phosphorylase enzymes in the liver and muscles, and subsequently the following task was set - to investigate the effect of collection on the rate of glucose formation from its precursors under conditions of hyperglycemia.

To elucidate the mechanism of action of mulberry and psyllium polysaccharides on carbohydrate metabolism, it seemed necessary to study the activity of the phosphorylase and hexokinase systems in the above models of experimental diabetes.

Enzyme deficiency leads to inhibition of anaerobic glycolysis and, accordingly, to stimulation of gluconeogenesis. Therefore, in order to elucidate the mechanism of action of the collection, it seemed expedient to elucidate the state of the hexokinase system under conditions of diabetes.

Previously, in a series of experiments, it was found that a decrease in blood sugar levels in alloxandiabetic rats under the influence of fees is accompanied by a significant increase in glycogen in the liver and muscles. Hypoglycemic activity of a dry extract consisting of two plants *Morus alba*, *Plantago major* used in folk medicine for the treatment of diabetes mellitus (type II). In previous works, we published the results of a study of a dry extract of medicinal plants that have a hypoglycemic effect under conditions of experimental hyperglycemia [2]. The results obtained were compared with the hypoglycemic effect of oranil used in the treatment of diabetes [8].

The aim of this work was to determine the enzymes of hexokinase and phosphorylase in the liver and muscle tissue with the determination of the amount by the radioimmune method and to determine the effect of the collection on the intensity of gluconeogenesis in the liver tissues in normal and experimental diabetes.

Materials and methods of research: The object of the research was an extract of local plants - white mulberry leaves and leaves of the greater plantain (*Morus alba*, *Plantago major*).

The experiments were carried out on 12 white mature rats weighing 120-140 g, kept on a normal diet. Animals were divided into three groups of ten in each: In the first group, the state of carbohydrate metabolism was studied in the normal intact control (IC), the second group was control pathology (KP) animals with experimental diabetes injected with saline alloxan hydrate, the third group was control pathology (KP) - animals with experimental diabetes + local plant extract.

Experimental hyperglycemia was induced by a single intra-abdominal injection of a physiological solution of alloxan hydrate 17 mg/100 g per body weight (alloxan at a dose of 170 mg/kg). The development of diabetes was followed by an increase in the level of glucose in the blood not lower than 17-20 mmol/l, an increase in water intake and weight loss. [2,4]

The plant extract was administered to animals with alloxan diabetes once a day for 1,3,7 days at a dose of 50 mg/100 g and oranil at a dose of 100 mg/kg administered orally. Then, every subsequent 30 minutes for 2.5 hours, blood was taken from the tail vein and the concentration of sugar in the blood was determined by the enzymatic method (Table 1) [5].

The choice of the indicated dose and the timing of the study are due to the fact that pharmacologists studied the effect of the collection at this dose and at these times. Therefore, the indicators obtained by us during these periods served as a criterion for comparing our data with the results of the literature. The general condition of the animals was monitored for one week in a vivarium. Determining the content of sugar in the blood, the objectives of our work as research tests were to determine the activity of hexokinase and phosphorylase enzymes in the liver and muscles in experimental diabetes. The tests were carried out in normal conditions in intact animals, as well as in control and experimental animals with diabetes under the influence of the extract. After 7 days, the rats were decapitated and blood sugar levels were determined at 30-minute intervals, i.e., after 60, 90 and 120 minutes.

Determination of hexokinase activity in tissues was determined by the Neifach method [6] based on the decrease in glucose consumed for the formation of glucose-6-phosphate during the hexokinase reaction $\text{Glucose} + \text{ATP} + \text{hexokinase} \rightarrow \text{glucose-6-phosphate} + \text{ADP}$. Hexokinase activity is expressed in conventional international units (IU). Statistical processing of the results was carried out according to Fisher-Student, phosphorylase activity in the liver tissue, we used a method based on determining the decrease in inorganic phosphorus in the incubation medium under the influence of phosphorylases as a result of glycogen breakdown. The amount of inorganic phosphorus before and after incubation was determined by the Fiske-Subbaru method.

In accordance with the objectives of our work, studies were carried out to determine the intensity of gluconeogenesis in sections of hepatic tissue in the norm in intact animals, as well as in control and experimental animals with diabetes under the influence of hypoglycemic collection.

The rate of gluconeogenesis in liver tissue sections was determined [7] by incubation of liver sections in Krebs-Rieger bicarbonate buffer pH – 7.4 with the addition of one of the substrates (alanine, α -ketoglutaric acid, pyruvic acid, succinic acid) at a final concentration of 0.01 M. Incubation was carried out under aerobic conditions at 37°C and constant shaking for 1 h. The amount of glucose in the incubation medium was determined by the glucose oxidase method [5].

The rate of gluconeogenesis was expressed as mg of newly formed glucose per 1 hour per 1 g of raw liver tissue.

Results of the study: The influence of the collection (dry extract) of medicinal plants with a hypoglycemic effect in conditions of alloxan diabetes, on reducing the amount of sugar in the blood, was studied. The results showed that the collection of medicinal plants, in contrast to their individual extracts, contributes to a significant reduction in blood sugar. The decrease in blood sugar levels in rats with alloxan diabetes and the amount of some metabolites in the analysis were studied. And also after blood sampling to determine the amount of sugar in the blood, the rats were decapitated and the enzyme activity was studied. According to the results of the study, it was found that the collection has hypoglycemic activity and can be used as an antidiabetic agent for type II diabetes.

The results of the experiments showed that the collection with daily administration for seven days leads to a decrease in the level of sugar in the blood of diabetic rats by more than two times (Table 1). The entry of glucose into the reaction of energy metabolism of the cell is carried out through its primary phosphorylation with the participation of hexokinase in the liver and muscles. Phosphorylation is the main mechanism for the involvement of glucose in

metabolic processes. The results of experiments on the activity of enzymes in tissues with daily administration for 7 days are shown in Scheme 1 and in Table 2.

Table 1

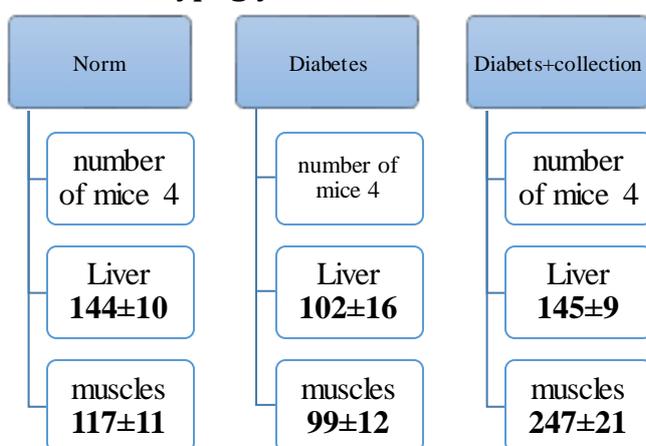
The content of sugar in the blood against the background of alloxan diabetes (n=12)

Indicators	Number of mice	Sugar mmol
Intact (norm)	4	5,4 ± 0,1
Control (diabetes)	4	19,2 ± 3,4*
Experience (diabetes+collection)	4	8,8 ± 0,9*

*- p < 0.001

Scheme 1

The effect of hypoglycemic collection on the activity of the hexokinase enzyme



*Reliability p < 0.05

The numerical data shown in the scheme show that the collection contributes to the stimulation of muscle hexokinase with repeated administration more than twice. In diabetes, there is a sharp decrease in hexokinase activity, despite the high content of sugar in the blood serum - a substrate for this enzyme. There is reason to believe that a change in the activity of hexokinase, which plays a major role in maintaining sugar hemostasis, occurs as a result of a change in the amount of the enzyme. The results obtained for the duration of incubation shown in the table (Table 2) clearly indicate a significant decrease in the activity of muscle phosphorylases in diabetic rats that received multiple collections.

Table 2

The effect of hypoglycemic collection on the activity of the enzyme phosphorylase

Mo reo ver , the mo	Experience conditions	Group	Sex	Number of Mice	Incubation duration	
					30 min	60 min
	Norm	1	Males	4	19,3±3,6	29,6±2,4
	Diabetes	2	Males	4	27,1±3,2	43,5±4,1
	Diabetes + collection	3	Males	4	19,9±2,5	31,2±5,2

st noticeable decrease (up to 30%) of enzyme activity corresponds to repeated administration of the collection. It is not possible to say anything definite about the mechanism for reducing the activity of phosphorylases at this stage of studying the effect of the collection.

In experiments, sugar at physiological concentrations activates glycogen synthetase and inactivates phosphorylase. The regulation of the activity of these enzymes by glucose is based on their cooperative interaction.

It is essential for this work that the hypoglycemic effect of a number of oral antidiabetic drugs, sulfonylurea and biguanides, is associated with their inhibitory effect on the processes of cAMP-dependent enzyme. Enzymes of gluconeogenesis are cAMP dependent, their state is important in the regulation of carbohydrate metabolism.

The above was the basis for the study of the gluconeogenic function of the liver in diabetes under the influence of the collection (table 3).

The main precursors of glucose formation in the liver are glycerol, amino acids and lactate. The results of experiments with perfused rat liver indicate that an increase in the plasma concentration of any of these precursors can lead to stimulation of gluconeogenesis [6,7]

Table 3
The state of gluconeogenesis in the liver of intact rats
(mg glucose/1g tissue/hour, n=12)

Group Options	Control	Experience	Change in %	P
Without substrate	0,566±0,060	0,488±0,490	-11	P>0,1
Alanine	0,622±0,041	0,507±0,022	-18	P>0,05
Pyruvate	0,623±0,092	0,563±0,057	-9	P>0,5
Succinate	0,634±0,050	0,603±0,044	-5	P>0,5
Ketoglutarate	0,630±0,021	0,612±0,046	-3	P>0,5

The increase in newly formed glucose, regardless of the nature of the substrate, with the exception of alanine, did not exceed the basal level. The absence of a noticeable increase in gluconeogenesis in intact animals corresponds to the literature, which shows that amino acids (aspartate, glutamate, propionate, etc.), as well as metabolites of the Krebs cycle (citrate, succinate, lactate, and α -ketoglutarate) slightly exceeded the control level of gluconeogenesis or completely did not affect its speed, because in liver sections, experiments to study the rate of glucose production from individual precursors were carried out, as a rule, at high concentrations of substrates and reflect the maximum rate of gluconeogenesis.

At the same time, the presence of alanine in the incubation medium showed a significant decrease in glucose concentration by 18% compared with rats that did not receive the extract. This is due to the fact that normally alanine occupies a special place in maintaining the level of glucose synthesized de novo, the carbohydrate skeleton of which is easily transformed into glucose in the liver. It is possible that under the action of the collection, the participation of alanine in gluconeogenesis is somewhat limited.

The established results served as a control in studying the effect of the collection on the rate of gluconeogenesis in diabetic conditions. From the materials of table 4 it can be seen that the collection is able to inhibit the rate of formation of glucose from its de novo precursors in the liver.

Table 4

The state of gluconeogenesis in the liver of rats with diabetes after the introduction of the collection (1 mg glucose / 1 g raw tissue / hour, n=12)

Group Options	Control	Experience	Change in %	P
Without substrate	0,572±0,054	0,412±0,048	-28	P<0,05
Alanine	0,615±0,044	0,387±0,042	-37	P<0,01
Pyruvate	0,650±0,066	0,562±0,058	-14	P>0,05
Succinate	0,632±0,038	0,502±0,052	-21	P<0,05
Ketoglutarate	0,640±0,021	0,458±0,033	-28	P<0,05

As can be seen from the table, when the drug is administered, a noticeable suppression of gluconeogenesis is observed, and the direction of changes is the same both without a substrate and with a substrate, especially if alanine is used as a substrate. This state is of particular interest in light of the role of alanine in carbohydrate metabolism, which is considered the key amino acid in the process of gluconeogenesis. It is known that the gluconeogenic action of amino acids in the body is under strict hormonal control, especially insulin, which is an antagonist of adrenaline in the regulation of gluconeogenesis. Insulin is the only hormone that inhibits the formation of glucose in the body by inhibiting all key enzymes of gluconeogenesis. Based on these considerations, it can be assumed that the inhibitory effect of the collection on gluconeogenesis is mediated through its action on insulin or glucagon.

In this regard, the totality of the presented materials indicates that the inhibition of gluconeogenesis under the action of the collection proceeds with a parallel stimulation of tissue sensitivity to insulin secreted by intact tissues in diabetes or recovery under the action of an extract of a dry hormone-receptor relationship with a simultaneous increase in glucose utilization in tissues.

It is essential for this work that the hypoglycemic effect of a number of oral antidiabetic drugs, sulfonylurea and biguanides, is associated with their inhibitory effect on the processes of cAMP-dependent enzyme. Enzymes of gluconeogenesis are cAMP dependent, their state is important in the regulation of carbohydrate metabolism.

The above was the basis for the study of the gluconeogenic function of the liver in diabetes under the influence of the collection. The main precursors of glucose formation in the liver are glycerol, amino acids and lactate. The results of experiments with perfused rat liver indicate that an increase in the plasma concentration of any of these precursors can lead to stimulation of gluconeogenesis [1,6]

Conclusions:

1. Detected can be considered as an insulin-like action of the collection. In diabetes, due to lack of insulin, which controls the synthesis of these enzymes, their activity is sharply reduced.
2. In conditions of alloxan diabetes, the collection led to a decrease in blood sugar levels by more than two times, which was accompanied by inhibition of the activity of tissue phosphorylases and significant stimulation of hexokinase in the liver and muscles.
3. In conditions of alloxan diabetes, the collection inhibits gluconeogenesis in the liver, which is especially pronounced in relation to alanine. In combination with epinephrine, the collection contributed to a significant decrease in the effect of adrenaline on the formation of glucose from non-carbohydrate precursors.
4. Our experiments show that in intact animals, gluconeogenesis in the liver tissue, assessed by the increase in glucose in the presence of various precursors, proceeded in the same way.
5. The results of the study and their analysis allow us to consider the local herbal preparation, which has a hypoglycemic property, as absolutely non-toxic when used orally.

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