ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

Anti-diabetic Activity of Dry Extract of Gymnema Sylvestre

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Abstract

The purpose of the study was to study the mechanism of antidiabetic action and compare the hypoglycemic effect of taking extracts of different concentrations of gurmar in a laboratory rat population. Studies were conducted in 2018 in a population of 400 laboratory rats based on the Federal State Autonomous Educational Institution of Higher Education I.M. Sechenov First Moscow State Medical University of the Ministry of Health of the Russian Federation (Sechenov University) (Moscow, Russian Federation). 300 animals made up the experimental group, 100 - the control. We used 25, 50, and 75% gurmar extract and diabeton, which were given orally to animals from the experimental group at a dosage of 70 mg to 1100 mg per 1 kg of body weight and 10 mg per 1 kg of body weight, respectively. Tests for glucose digestibility were performed by administering extracts of gurmar after fasting, dosage of 270 mg per 1 kg of body weight. The hypoglycemic effect of gurmar-based drugs activates enzymes associated with the inhibition of absorption of glucose from the intestine, as well as with its breakdown. Studies have established 25% as the most optimal dose of gurmar extract. The dosage in this case is 270 mcg per 1 kg of body weight. The maximum hypoglycemic effect is observed 2 hours after taking the drug. For high concentrations of the drug, a 25–30% lower hypoglycemic activity was recorded. Another drug, taken for comparison-diabeton, is superior in its effect to 25% gurmar extract by 20%. A 30% increase in beta-endocrinocyte nuclei may indicate an increased physiological activity of these cells.

Keywords: *Gurmar, type 2 diabetes mellitus, Extracts, Diabeton.*

Introduction

Diabetes mellitus is one of the most dangerous diseases of our time [1]. Mortality due to diabetes can reach 6 people per 100 thousand of the population. Some people remain permanently disabled - every 2-3 people per 100 thousand of the population [2]. Among the consequences of diabetes mellitus, a wide variety of diseases appear - coronary heart disease, nephropathy, retinopathy [3].

Conventional methods for treating diabetes mellitus, especially type 2diabetes, are not always effective and may not have a sufficient therapeutic effect on blood glucose levels [4]. In recent years, diabetes therapy has undergone significant changes. If earlier the main goal was the fight against hyperglycemia, then in modern medicine the main attention is paid to the search for methods of protecting pancreatic islets of Langerhans islets from depletion, as well as preventing vascular complications at the macro and micro levels [5]. The increasing number of cases of diabetes mellitus in recent years has forced the search for new drugs that can have a hypoglycemic effect [6]. In particular, in the treatment of type 2 diabetes mellitus, GLP-1 (glucagon-like peptide-1) preparations are used. This peptide has an incretin-mimetic effect, but is not effective enough due to the low resistance of its synthetic analogue [7].

Therefore, a further search for drugs against diabetes is always relevant. The basis of diabetes influence is manifested, first of all, in terms of blood microcirculation disorders. Hyperglycemia triggers the oxidation of lipid peroxides [8]. The consequence of this is the observed changes in the structure and permeability of the RBC (red blood cells) membranes. These changes are associated with changes in lipid composition [9].

At a functional level, an increase in an indicator such as viscosity occurs in the inner layer of lipids of the erythrocyte membrane [10-13]. Angiopathies that are observed at the micro and macro levels lead to disruption of the body's normal homeostasis [14]. All of the above pathological changes can be mitigated at an early stage, in case of diagnosis, including through pharmacotherapy [15].

The drugs used for diabetes treatment include those created on the basis of the gumnar extract (DIA-ß drug). Gumnar (*Gymnema sylvestre*) is a plant from the *Apocynaceae* family that grows in the forests of the tropical part of Asia, Africa, as well as in the forests of Australia. The plant in Ayurveda's traditional medical practice is used as a sugar-reducing agent (translated from Indian as sugar killer) [5]. According to reports, the therapeutic effect of gurmar as a sugar-lowering plant is based on the release of insulin [10]. Another therapeutic effect of anthem is expressed in the regeneration of ß-cells located in the physiologically active part of the pancreas, the so-called islets of Langerhans [11, 12].

In addition to all of the above, there is a decrease in the level of absorption of glucose in the intestinal lumen, and activation of enzymes that break down glucose in the tissues of the body [16]. It is also known that gumnar extract is able to effectively lower blood lipids (triglycerides and cholesterol) [2]. The available studies are mainly devoted to the physiological mechanisms of the effect of gumnar extract on the protein-carbohydrate and lipid metabolism in the body [17, 18, 5].

But for a full analysis of these mechanisms and the effect of gurmar-based drug therapy, an understanding of the effect of these drugs on blood sugar concentration is necessary. This is the main subject of this study. The aim of this work is to study the mechanism of antidiabetic action and to identify the most effective concentrations of forest hymnoma extract on the material of laboratory rat population. The subject of the study is the hypoglycemic effect, the object of the study is the laboratory population of rats.

Material and Methods

Material

The studies were conducted in 2018 on the basis of the Federal State Autonomous Educational Institution of Higher Education I.M. Sechenov First Moscow State Medical University of the Ministry of Health of the Russian Federation (Sechenov University) (Moscow, Russian Federation).

For the experiment, 300 (experimental group) and 100 (control group) of sexually mature outbred laboratory white rats weighing 300-320 g were selected. Before the experiment, the rats were kept in vivarium conditions and were fed according to established standards, balanced in terms of vitamins and minerals, carbohydrates, proteins and fats with food (standard GOST P50258 - 92).

The experiments were carried out according to the standards of preclinical laboratory research (GOST Z51000.3-96, as well as 51000.4-96).During the experiment, the ethical and moral rules of scientific ethics was not violated. For research, certified dry extracts of forest anthem were selected (certificate GYM-030706 dated 06.2003), with gravimetric concentrations of 25, 50 and 75%, respectively. Extracts producer -SCS "Alliance" (Russian Federation).

Research Methods

A single oral administration of the gumnar medication was carried out, with a dosage of 70 mg to 1100 mg per 1 kg of body weight. Determination of sugar level was carried out by taking blood from the tail vein of rats. Sugar concentration was determined using the enzymatic method using the Glucose FKD kit (Russian Federation). Glucose tolerance tests were carried out by administering gumnar extracts fasted, with a dosage of 270 mg per 1 kg of body weight. After 1 hour after administration of the extract, 3 g of glucose per 1 kg of body weight was administered intragastrically.

Subsequently, blood glucose was determined every 20 minutes, within 2 hours after the introduction of sugar. To compare the effect of the gurmar extract on blood sugar levels, another drug was used, diabeton (manufacturer - Servier, France, European Union).The dosage of diabeton was 10 mg per 1 kg of body weight. On an isolated rat diaphragm, we assessed the glucose uptake by peripheral tissues. The rats were injected with an aqueous solution of gurmar extract for 1 month (30 days), with a dosage of 270 mg per 1 kg of body weight. 15 hours before the start of the experiments, the rats

underwent deprivation. At the same time, animals had free access to drink. Rats were decapitated, after which the diaphragm was removed. After extraction, the diaphragm was washed in physiological saline, put on ice, and was divided into two equal parts.

Subsequently, one part of the diaphragm was placed in a container with a buffer (Krebs-Ringer bicarbonate buffer, pH = 7.4). The amount of glucose in the buffer is 11 mmol per 1 liter. The second part of the diaphragm was placed in a similar buffer, but it also contained insulin, at a concentration of 0.05 U per 1 ml. Next, a two-hour incubation of both diaphragms was carried out in a thermostat at a temperature of 37°C.

Further, after incubation, the residual glucose concentration was determined using the glucose oxidase method. Based on the results obtained, the glucose uptake coefficient in mg per unit mass of the diaphragm (g) was evaluated. We have also evaluated the effect of the extract of forest anthem on the functional state of cells in the islets of Langerhans in the pancreas. To do this, extract was introduced into the stomach for 1 week at a dosage of 270 mg per 1 kg of body weight. When the experiment was completed, pancreatic tissue was evaluated by morphological methods. The pancreatic tissue was divided into three parts, corresponding to the duodenal, gastric and splenic fragments. Tissues were stained with hematoxylin as well as eosin. Parameters such as the area of the Langerhans islands (in μm^2), as well as the area of the nuclei of beta-endocrinocytes (also in μm^2) were evaluated. The volume fraction occupied by the islets of Langerhans was calculated in %.

Statistical Analysis

Processing of the results was carried out using the Past v.3.0 software. We calculated the main statistical indicators, such as the arithmetic average, the error of mean. We used parametric methods of statistical analysis (paired Student's t-test), since the distribution of data corresponded to normal.

Results

The most effective action of the gumnar drug is in 25% concentration (Fig. 1).

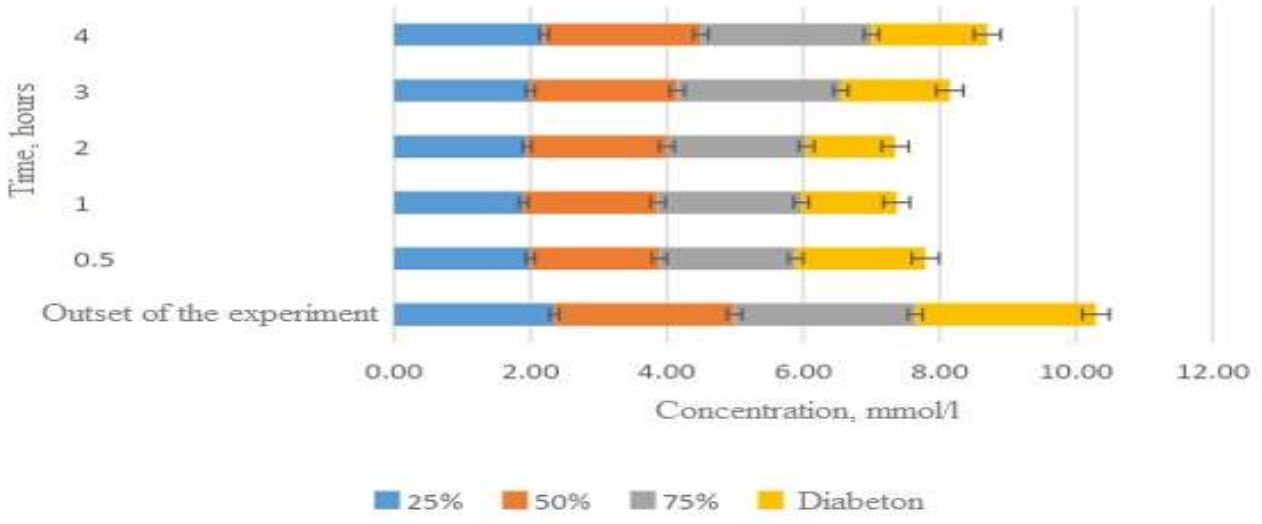

Figure 1: The effect of dry gumnar extract (at concentrations of 25, 50, 75%) and diabeton on the concentration of glucose in rat blood

After 2 hours, the effect of the drug in this concentration is manifested. The gumnar drug in other concentrations (50 and 75%) showed less effectiveness - the decrease in glucose levels at the beginning of the experiment by 4 hours after the injection almost reached the experimental concentration. Diabeton showed the maximum efficiency - the average glucose concentration during the experiment was 1.76±0.19 mmol per 1 liter, whereas for the gumnar extract these indicators are higher: 2.07±0.10 mmol per 1 liter for 25%, 2.17±0.15 mmol per 1 liter for 50 %, and finally, 2.28±0.13 mmol per 1 liter for 75% extract. Thus, diabeton is 1.17 times more effective than 25% of the gumnar extract (the difference is not significant), 1.25 times - 50% of the extract, and 1.3 times - 75% of the extract ($p \leq 0.05$). It was found that the effect of 25% of the extract begins already after half an hour, reaching its maximum for lowering

glucose levels after 2 hours, by 30% lowering the level of glucose ($p \leq 0.05$). For a 50% extract, the decrease was 12%, but after 4 hours the glucose level was comparable to the control. For 75% of the extract, the maximum effect was noted after 2 hours, but with a hypoglycemic activity lower by 26%. We found a relationship between a decrease in blood glucose concentration and a dose of 25% of the extract (Fig. 2A). This dependence is fixed in the range of 70 - 270 mg per 1 kg.

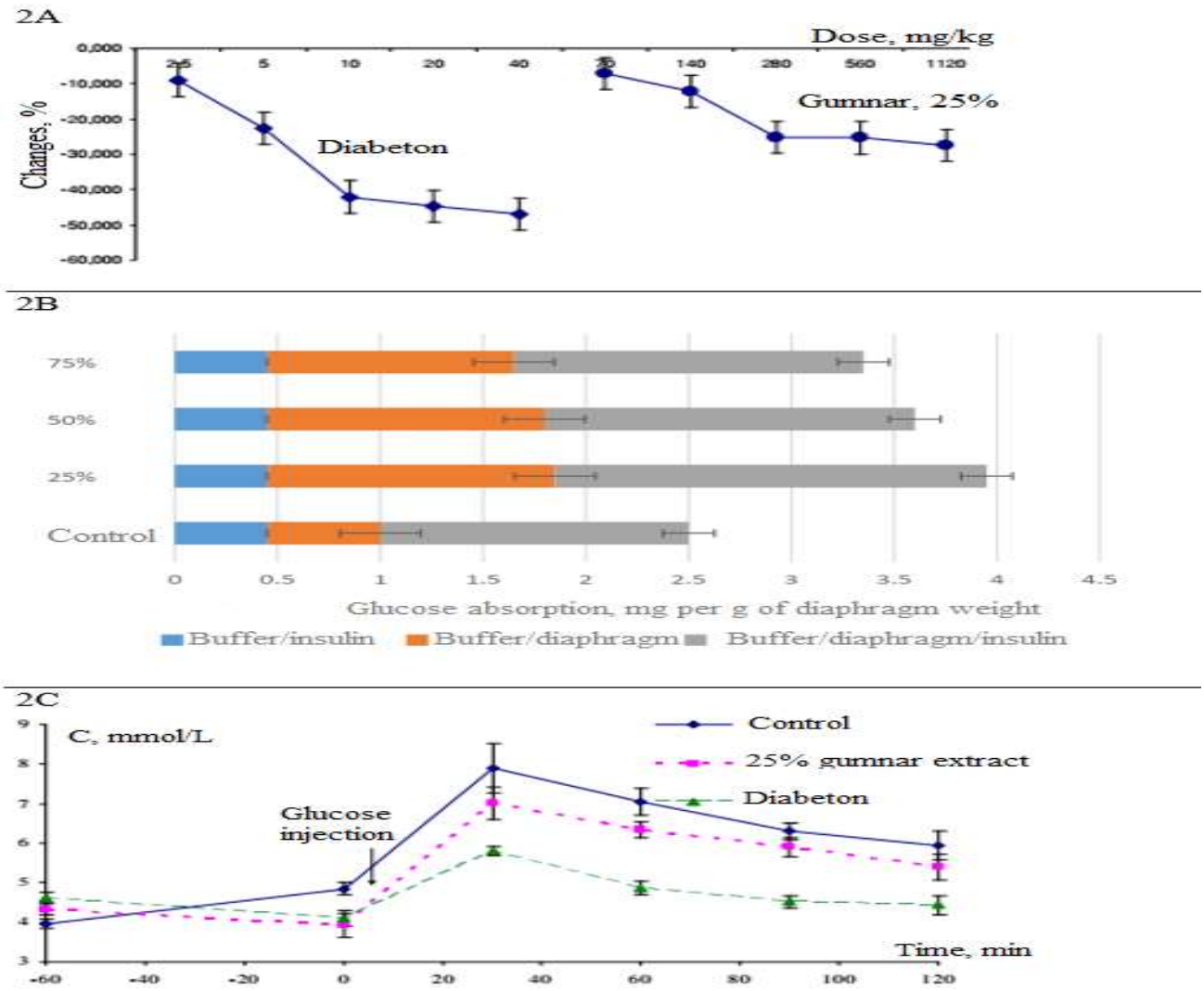

Figure 2: Hypoglycemic effects of gumnar extracts of different concentrations (25, 50, 75%) and diabeton 2 hours after injection (2A), absorption of glucose by peripheral tissues (2 V), and the effect of the extracts orally after glucose loading (2 C)

The minimal hypoglycemic effect was noted at a dose of 70 mg per 1 kg (6.9%), but already with a doubling of the dose, the effect doubles to 13.1% ($p \leq 0.05$). Another increase in the hypoglycemic effect was noted when the dose was doubled to 270 mg per 1 kg (25%, $p \leq 0.05$). A further increase in dose did not give a significant increase in the hypoglycemic effect.

In this regard, we worked further with a dose of 270 mg per 1 kg of body weight, with 25% gumnar extract. In the tissue of the diaphragm of animals that received a 25% gumnar extract, 2 times higher glucose uptake rates were observed compared with the control (p $p \leq 0.05$) (Fig. 2B). For a solution containing insulin, glucose decreased by a third compared to the control ($p \leq 0.05$).Next, we examined the absorption of glucose without the presence of insulin, for 50 and 75% of gumnar extracts. If for 50% of the extract the glucose level decreased by 15%, then for 75% it decreased by 10% in relation to the control. From this it can be concluded that glucose in the presence of the anthem preparation can be absorbed intracellularly.

Already half an hour after the injection of glucose at a dose of 3 g per 1 kg of body weight, its concentration was maximum, with a twofold excess of the initial values (Fig. 2 C). After this, a gradual decrease in glucose concentration occurred, with reaching values close to the original 2 hours after the

injection. After the injection of diabeton and 25% of the gumnar extract, forest hyperglycemia decreases in its indices by 15 and 25%, respectively ($p \leq 0.05$). From this it follows that the hypoglycemic activity of the gumnar extract is 25% less in comparison to the control. This may mean that the action of 25% of the extract is aimed at the absorption of carbohydrates in the intestine, as well as in the muscles, as was shown by the example of the diaphragm.

Morphological studies of the islets of Langerhans showed that in the control animals and rats treated with 25% gumnar extract, there was uneven plethora of microvessels-capillaries (Fig. 3A). A moderate degree of increase in beta-endocrinocytes nuclei was also noted; in all three samples, it was 1.2-1.3 times in the group receiving 25% extract compared to the control.

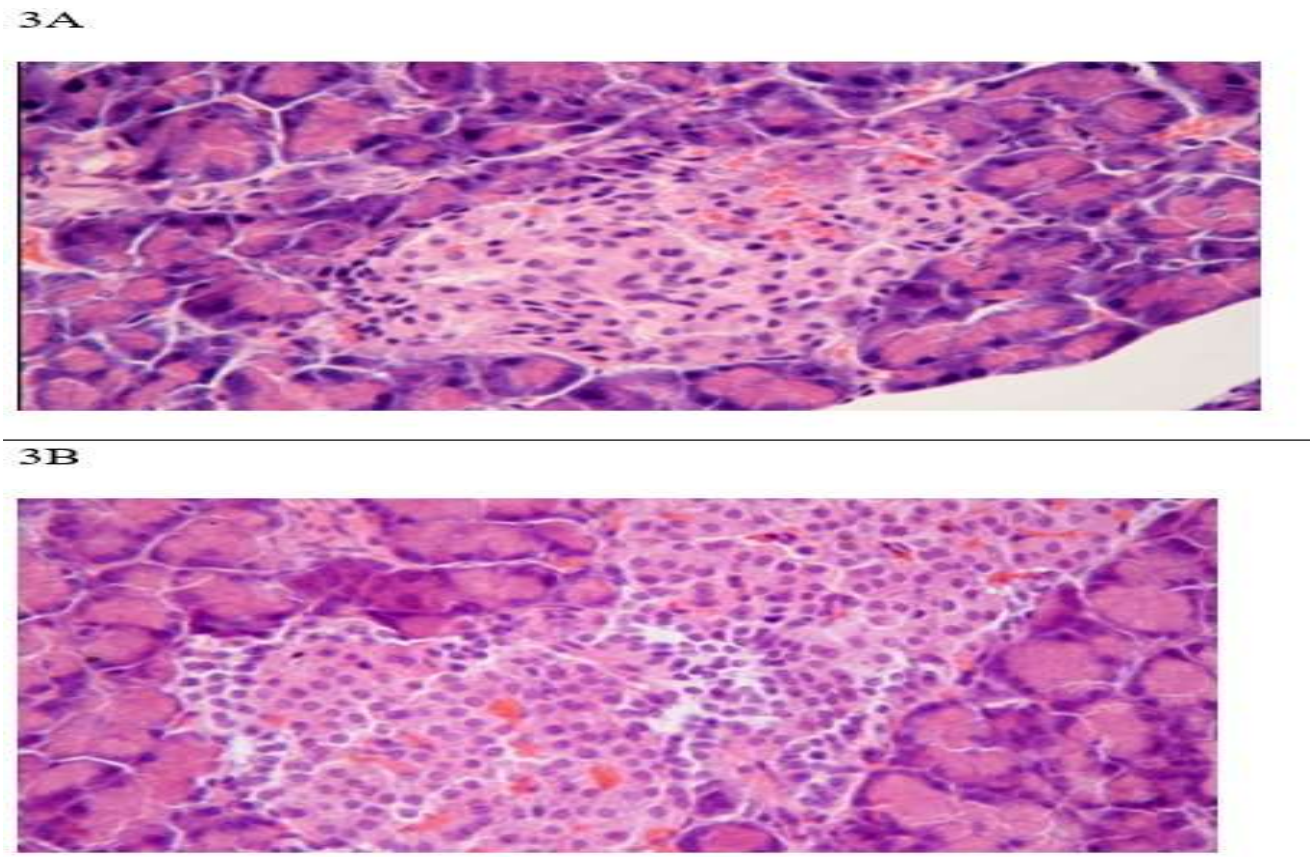

Figure 3: Islets of Langerhans in the control (3A) and in the group of rats treated with 25% gumnar extract (3B)

Nevertheless, we obtained significantly different results in the sizes of pancreatic nuclei in the control and experimental groups (Table 1). An increase in the size of the nuclei of cells indicates their increased physiological activity. Also there was an increase in such characteristics as the volume fraction and the area of the islets of Langerhans in the experimental and control groups (Table 1). But, at the same time, the morphometric parameters did not bear significant differences. Thus, the area of pancreatic nuclei turned out to be the most reliable indicator.

Table 1: Characteristics of the islets of Langerhans in control and experiment

Groups	Duodenal pancreas		
	Proportion of islets, %	Area of islands, μm sq	Area of pancreatic nuclei, μm sq
Control	6.7±1.9	40.7±14.6	22.7±3.8
Experiment	7.2±2.4	62.1±21.9	30.9±1.7*
	Gastric pancreas		
Control	12.1±4.1	52.1±31.2	24.1±2.9
Experiment	13.5±4.5	100.1±45.1	30.3±1.1*
	Splenic pancreas		
Control	14.0±7. 6	52.3±21.1	25.3±2.0
Experiment	15.1±8.2	129.3±78.1	32.5±2.0 *

*-differences are significant at $p \leq 0.05$
An excess of control indices by a third ($p \leq 0.05$) from the experiment was recorded

Discussion

We have found that the use of 25% gumnar extract causes activation of glucose uptake. Some evidence suggests that the hypoglycemic effect of gumnar is associated with activation of enzymes that are associated with inhibition of glucose uptake from the intestine, as well as with its breakdown [19-23]. Perhaps this is due to increased insulin secretion by beta-endocrinocytes.

Such data were obtained by other authors for alcoholic gumnar solutions [24-26]. About 60% of the insulin produced by the pancreas is secreted under the influence of GLP-1, as a response to oral glucose (Zhu et al., 2008; Reddy et al., 2012). It is also known that gumnar extract releases GLP-1, thus indirectly contributing to the hypoglycemic effect (Sarkar et al., 2009). Our data also confirmed this hypothesis - but adjusted for the maximum efficiency of 25% gumnar extract. The action of gumnar drugs manifests not only at the physiological level, but also at the morphological level - the sizes of the nuclei of pancreatic cells change, as was shown by our data. This direction seems to us quite relevant and effective, with the possibility of further research.

Conclusions

Studies have established 25% as the most optimal dose of gumnar extract. The dosage in this case is 270 mcg per 1 kg of body weight.

The maximum hypoglycemic effect is observed 2 hours after drug administration. For high concentrations of the drug, a hypoglycemic activity lower by 25-30% was recorded. Another drug, taken for comparison - diabeton, is 20% superior in its effect to 25% gumnar extract. A 30% increase in beta-endocrinocytes nuclei may indicate an increased physiological activity of these cells.

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