Correction of cognitive function of laboratory rats with diabetes with extracts of nettle and ginkgo biloba

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Abstract. Scientists have objectively demonstrated memory and attention impairments in patients with diabetes mellitus (DM). Currently, neuropsychological testing of patients with diabetes reveals more pronounced impairments in cognitive functions. In the treatment of diabetes mellitus, the use of low-toxic hypoglycemic drugs of plant origin is effective, which not only reduce blood glucose levels, but also have a pleiotropic effect, in particular, normalize cognitive functions. The study showed the optimal effect of nettle extract and ginkgo biloba to normalize cognitive impairment in laboratory rats with hyperglycemia. 3-month-old laboratory white male rats weighing 250-300 g, nettle extract (natural, organic, healthy products), ginkgo biloba extract (Leovit) were used as the object of the study. A test was conducted in a T-shaped maze evaluating the rats' learning ability and working memory, an outdoor test evaluating motor activity and spatial orientation, an adhesion test evaluating tactile sensitivity and fine motor skills, and a balloon burying test.

1 Introduction

Scientists have objectively demonstrated memory and attention disorders in patients with diabetes mellitus (DM). Currently, neuropsychological testing of DM patients reveals more pronounced cognitive impairment. In the treatment of diabetes mellitus, the use of low-toxic hypoglycemic drugs of plant origin is effective, which not only reduce blood glucose levels, but also have a pleiotropic effect, in particular, normalize cognitive functions.

According to the International Diabetes Federation, the number of diabetic patients worldwide was 424.9 million in 2017. Worldwide, diabetes is a huge public health problem and is pandemic in nature [1].

According to the World Health Organization, the National Register of Diabetic Patients in Kazakhstan has been maintained since 2002. According to the statistical data of the Diagnostic and Statistical Manual information systems, 382 thousand patients were registered in Kazakhstan at the beginning of 2021.

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Of these, 30 thousand are patients with type I diabetes mellitus, 352 thousand are type II, and 431,871 people were registered for dynamic control in the 1st quarter of 2022 [2-4].
In recent years, a large amount of data has been accumulated on the relationship between cognitive impairment (CI), dementia and diabetes mellitus (DM).

Study of the combined effect of nettle and ginkgo biloba extracts: Previously, studies on the correction of cognitive functions in diabetes mainly focused on individual extracts, rather than on their combination. This work explores the synergistic effect of these two extracts [5].

Using a laboratory model of diabetes: Most previous studies have been conducted on animal models that do not fully mimic diabetes in humans. In this work, a model of diabetes in rats is used, which is more similar to a human disease. Comprehensive assessment of cognitive functions: The study will evaluate a wide range of cognitive functions, including memory, attention, executive functions and spatial orientation. This will provide a more complete understanding of the effect of extracts on cognitive impairment in diabetes [6-8].

Mechanistic approach: The work will explore the mechanisms underlying the cognitive improvements caused by extracts. This includes measuring levels of neurotransmitters, inflammatory markers, and growth factors in the brain.

Potential therapeutic applications: The results of the study may have significant therapeutic applications for people with diabetes suffering from cognitive impairment. The successful development of effective and safe methods of cognitive correction can improve the quality of life and independence of this vulnerable population group[9,10].

Purpose of the study: To evaluate cognitive functions of laboratory rats with extracts of nettle and ginkgo biloba in experimental diabetes.

2 Materials and Methods

The experiments were performed on 40 – 3-month-old white male laboratory rats weighing 300±50 g. The rats were given oral extracts from nettle (NOW foods 250 mg) and ginkgo biloba (Leovit 100 ml) plants. The studied animals were divided into 4 groups: 1-control, in animals of groups 2, 3, 4 diabetes was caused by intraperitoneal injection of streptozotocin at a dose of 65 mg / kg of weight, after 7 days, groups 2,3 and 4 were orally administered phytopreparations of nettle and Ginkgo biloba separately and in combination for 40 days. The studied extracts of nettle root (NOW foods) and the drug Leovit in effective therapeutic doses in the form of an aqueous suspension (mixed with water) were injected into the stomach daily 30 minutes before meals for 40 days of the study. Effective doses: for nettle root extracts — 50 mg /kg body weight, for ginkgo biloba extract — 100 mg /kg body weight. Control animals with a model of diabetes mellitus were injected with purified water in equal amounts. (Approval of the Local Ethics Committee of the Kazakh National University Protocol №IRB-A695 dated 11/09/2023). The results obtained were subjected to statistical processing, determined by Student's t-test.

Table 1. Experimental groups

Experimental groups	Groups	Used liquids	The days of the experiment
Control (intact)	1	Distilled water	40 days
Diabetes mellitus (experimental group)	2	Distilled water	40 days
Treatment (experimental group)	3	Nettle extract	40 days
	4	Ginkgo biloba	40 days
	5	Nettle + Ginkgo biloba	40 days

The methodology for creating an experimental model of diabetes and biochemical methods were used. The “T-maze” test was conducted to assess learning ability and working memory, the "open field" test to assess motor and orienting-exploratory behavior, the adhesive test to assess tactile sensitivity and fine motor skills, and the ball burying test – is used to determine a malfunction of the nervous system, in which healthy rodents bury part of the balls and calm down, and animals in the OCD model cannot stop until they bury everything to the last.

In the research work, experimental diabetes was caused by a single intraperitoneal administration of streptozotocin (50 mg/kg). During the 12 hours (nights) before the injection, the animals were not given food, there was free access to water. Streptozotocin was diluted with 0.4 ml of citrate buffer. After 72 hours administration of streptozotocin, blood glucose levels from the caudal vein were measured using a portable glucose meter (Sinocare Safe-Accu, China). After receiving the results, the animals were divided into the following groups of 10 rats according to the principle of randomization (Table 1).

3 Results and Discussion

Visual Assessment of glycemic levels in animals with experimental diabetes. On the 14th day of the study, PGTS was performed, as a result of which the glycemic level in the group with diabetes mellitus increased by 29.3% 30 minutes after glucose administration and remained at a high level after 2 hours (11.9±2.3 mmol/l), exceeding the baseline level by 9.2% (Table 2). As a result of pgts performed on days 28 and 40, there was a pronounced violation of glucose tolerance: 30 minutes after loading with glucose, the glycemic level increased by 36% and 34%, respectively. Blood glucose levels remained significantly high 2 hours after glucose loading (11,5±2,0; 12,3±2,0), from the entry level, by 12% and 14%, respectively.

Table 2. Assessment of blood glucose levels in rats that were not treated for 14, 28, 40 days in animals with experimental diabetes mellitus, mmol/L.

The days and time of the experiment		Control	DM
14 days	0 min	5,0±0,2	10,9±2,0*
	30 min	6,1±0,1	14,1±3,3*
	60 min	5,9±0,2	12,5±3,2*
	120 min	5,6±0,1	11,9±2,3*
28 days	0 min	5,1±0,2	10,3±2,3*
	30 min	7,2±0,2	14,0±3,2*
	60 min	6,4±0,1	13,6±2,5*
	120 min	5,7±0,1	11,5±2,0*
40 days	0 min	4,9±0,2	10,8±2,1*
	30 min	5,9±0,1	14,5±2,2*
	60 min	5,7±0,1	13,1±2,3*
	120 min	5,4±0,1	12,3±2,0*

Note: * Significantly compared with the control - $p < 0.05$*

Evaluation of the effect of nettle extract and ginkgo biloba on the glycemic level of rats with experimental diabetes. As a result of an oral glucose tolerance test conducted during the 14 days of the study, the glycemic level in the groups of diabetes + nettle, diabetes + Ginkgo biloba and diabetes + Nettle + Ginkgo biloba decreased by 35%, 30% and 42%, respectively, 2 hours after glucose administration, compared with the group that did not receive treatment. As a result of PGTS performed on day 28, the level of glycemia in the groups diabetes mellitus + nettle, diabetes mellitus + Ginkgo biloba and diabetes mellitus + nettle, + Ginkgo biloba decreased 2 hours after glucose administration by 44%, 32% and 48%, respectively, compared with the untreated group. As a result of PGTS performed on day 40, the level of glycemia in the groups diabetes mellitus + nettle, diabetes mellitus + Ginkgo biloba and diabetes mellitus+nettle, +Ginkgo biloba decreased by 49%, 37% and 53%, respectively, 2 hours after glucose administration, compared with the untreated group (Table 3).

Table 3. Blood glucose levels of rats treated during 14, 28, 40 days of the experiment, mmol/L.

The days and time of the experiment		Control	DM	DM + Nettle	DM + GB	DM + Nettle + GB
		5,8±0,2	12,6±0,9*	11,8±1,2*	12,1±1,5*	12,7±1,5*
14 days	0 min	5,0±0,2	11,1±2,3*	7,4±1,9*	7,5±1,4*	6,9±1,8*
	30 min	6,1±0,1	15,3±2,1*	10,9±2,1*	11,3±2,0*	10,1±1,3*
	60 min	5,9±0,2	13,5±2,3*	8,6±1,7*	9,3±2,1*	8,5±1,5*
	120 min	5,6±0,1	12,2±2,4*	7,9±1,1*	8,5±1,3*	7,1±0,8*
28 days	0 min	5,1±0,2	11,6±0,9*	7,0±1,3*	7,4±1,5*	6,0±0,9*
	30 min	7,2±0,2	15,4±2,2*	10,4±2,0*	11,0±2,1*	9,9±1,6*
	60 min	6,4±0,1	14,1±1,4*	8,1±1,6*	9,1±1,5*	8,1±1,1*
	120 min	5,7±0,1	12,3±1,2*	6,9±1,2*	8,3±1,3*	6,4±1,1*
40 days	0 min	4,9±0,2	11,5±1,7*	5,9±1,6*	7,1±1,9*	5,5±1,2*
	30 min	5,9±0,1	14,9±2,1*	9,9±1,9*	10,6±2,1*	9,3±1,5*
	60 min	5,7±0,1	13,7±1,3*	7,6±1,4*	8,2±1,2*	7,2±1,3*
	120 min	5,4±0,1	12,4±1,1*	6,3±1,0*	7,8±1,5*	5,8±0,9*

Note: * Significantly compared with the control - p <0.05*

Assessment of the motor and exploratory activity of rats treated and not treated in an outdoor test. According to the results of the open field test (Figure 1), in the group that did not receive treatment, motor activity decreased by 13% by 28 days, orientation and research activity- by 22%, which indicates a decrease in the reaction of animals to innovations and increasing apathy.

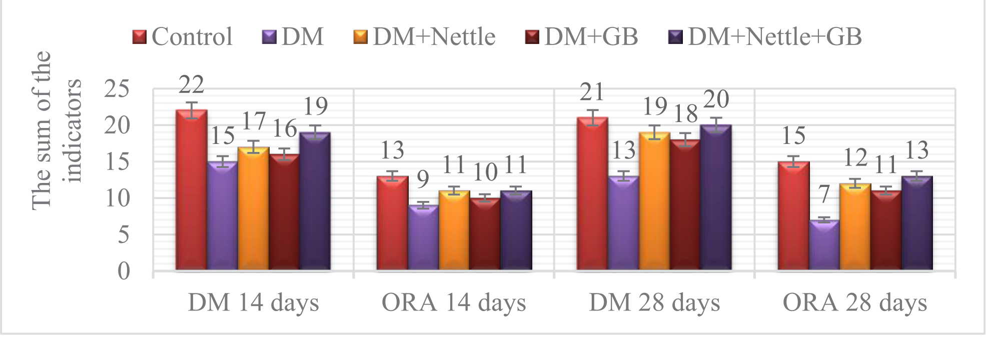

Fig. 1. Open-air test control, diabetes insipidus group and motor activity (MA) and orientation-research activity (ORA) of animals treated, %

28 days compared to 14 days the estimated research activity increased only in the control group and the groups of diabetes +nettle and diabetes+nettle +Ginkgo biloba treated, and in the group of diabetes+Ginkgo biloba-14% of the control group, diabetes +nettle – 5% of the group of diabetes +nettle+Ginkgo biloba - It was 10% less.

Assessment of tactile sensitivity and fine motor skills of rats treated and not treated with an adhesive test. The time spent on detecting a foreign body and getting rid of the sticky patch (Figure 2) was the highest among all animals in the untreated group and the diabetes+Ginkgo biloba group for 14 and 28 days, indicating progressive disorders of sensitivity and fine motor skills, as well as work disorders the nervous system. In the rats of the control group, these indicators practically did not differ. And the indicators in the diabetes+nettle+Ginkgo biloba group treated were 9% higher on day 14 and 8% on day 28 than in the diabetes+nettle group.

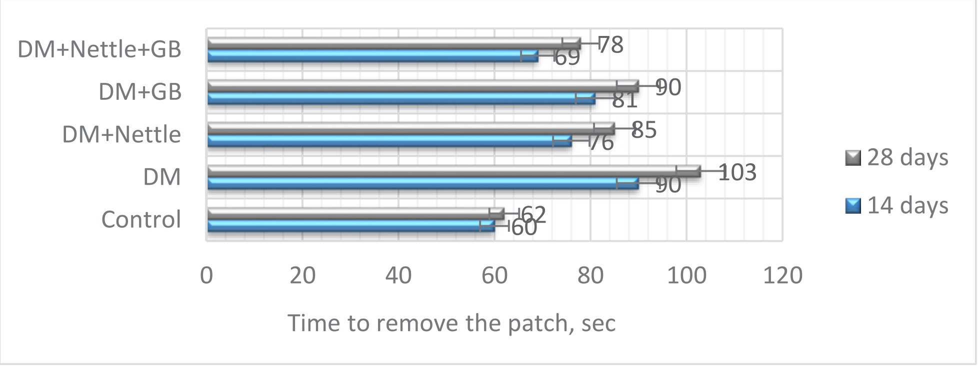

Fig. 2. Control, the result of an adhesive test on the untreated diabetes group and on the treated rats. Time to remove the adhesive patch, sec

Assessment of the protective behavior of rats treated and untreated during the balloon instillation test. The balloon burial test is traditionally considered as a model of obsessive-compulsive disorder, and may also indicate the level of anxiety in animals. In the test for instillation of glass beads on the 14th and 28th days after the start of treatment, the largest number of stationary beads was observed in the diabetes group+Ginkgo biloba and the smallest number of balls moved from the initial position (Table 4), and the lowest rates were observed in the group of diabetes who did not receive treatment, which, in turn, led to a decrease in motor activity and protective behavior of rats shows.

Table 4. The results of the adhesive test (piece)

Tests		Control	DM	DM + Nettle	DM + GB	DM + Nettle + GB
14 days	The number of balls moved	12	9	11	10	11
	2/3 number of buried balls	7	3	5	4	6
	Number of stationary balls	1	8	4	6	3
28 days	The number of balls moved	10	11	12	11	13
	2/3 number of buried balls	8	2	4	4	5
	The number of balls that are not in motion	2	7	4	5	2

The results of the assessment of learning ability and working memory of rats in control and experimental groups in the T-maze. The number of untreated rats that found food in the T-maze was 2 times higher during the 14, 18, 28, 32 days of the study compared with the diabetes group.The DM+nettle+GB group was higher compared to rats in another experimental group who had at least one food detection in 10 attempts, i.e.: 11% per day compared to the DM+nettle group and 67% higher compared to the DM+GB group. A decrease in the proportion of rats that found food in the T-maze indicates a violation of long-term memory in rats in the diabetes group who did not receive treatment (Figure 3).

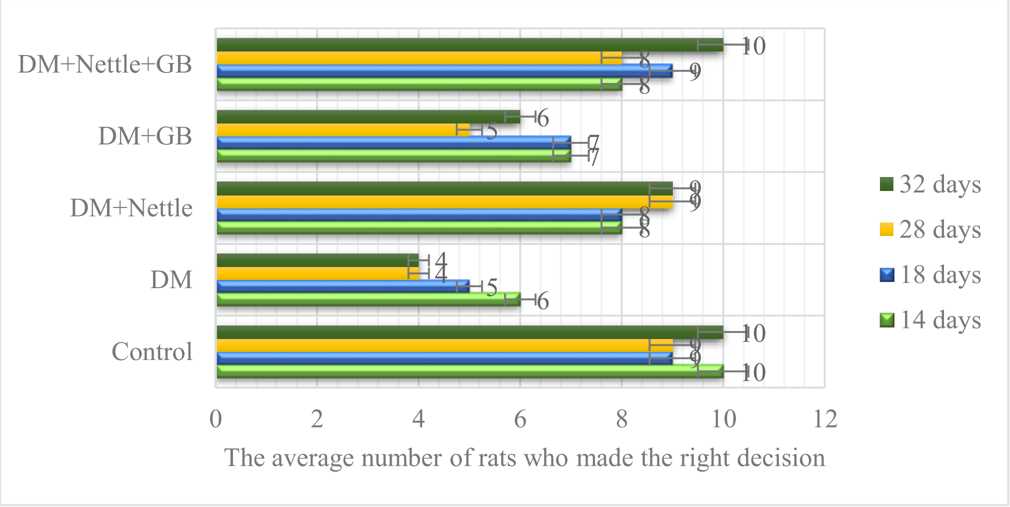

Fig. 3. The results of the assessment of learning and working memory of rats who received and did not receive treatment in the T-maze. (According to the ordinate: experimental groups; according to the abscissa, the number of skills in finding food for animals through the test (piece)

Experimental rats with diabetes showed that glycemic levels decreased due to nettle extract and ginkgo biloba, including an extract obtained from their combination, showed a greater effect. Evaluation of the motor and orientation-research behavior of rats in the outdoor nettle test and their combination were more active in the groups in which they were used. Assessing the tactile sensitivity and fine motor skills of rats using an adhesive test, nettle and ginkgo biloba show that they were the highest among all animals in the extract group, which led to a progressive disorder. Evaluation of the protective behavior of rats during the balloon burial test, as well as the ability to learn and work memory in the T-maze, the decrease in results associated with the use of nettle and ginkgo biloba extract is considered as a model of obsessive-compulsive disorder and anxiety levels in animals and indicates an increase in long-term memory.

4 Conclusion

The effect of herbal preparations on impaired cognitive functions in diabetes mellitus was shown, in the work the effect of ginkgo bioloba extract showed a positive effect on memory, concentration and attention stability, as well as psychomotor functions. The results of the study conducted with dioecious nettle allowed us to conclude that further research of nettle extracts as a means for the prevention of complications of diabetes mellitus is promising. In general, the combined action of two phytopreparations reduces memory and attention disorders, improving overall cognitive status and maintaining a normal balance of the body in diabetes mellitus. The action of phytopreparations has a pronounced antioxidant effect..

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