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The Effect Of Polyphenols On Hemostasis System Disorders In Rats With Experimental Diabetes

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ABSTRACT

Hemostasis disorders in patients with diabetes mellitus include the activation of natural coagulation mechanisms, suppression of fibrinolytic activity, and platelet dysfunction. In this regard, investigating this issue and searching for new pharmaceutical agents that can prevent the progression of type 2 diabetes mellitus and the development of its complications in the hemostasis system is a highly relevant problem.

Our studies have revealed that a polyphenol extracted from ***Pistacia vera*** and an extract from ***Isatis tinctoria L.*** possess antidiabetic and antithrombotic activity. It was demonstrated that these polyphenols improve glycemic control in rats with experimentally induced type 2 diabetes and also reduce the incidence of thrombotic complications.

Key words: Type 2 diabetes mellitus, modulation of diabetes, hemostasis system, plasma and biochemical blood parameters, polyphenols.

Introduction

In type 2 diabetes mellitus, virtually all components of the hemostasis system are affected, leading to disorders in coagulation, fibrinolysis, the anticoagulant system, and platelet function [1]. These factors contribute to the progression of atherosclerosis and cardiovascular diseases [2]. The main causes of these changes include insulin resistance, hyperinsulinemia, and poor glycemic control [3].

Currently, medications such as Diabeton MR, metformin, and others are used to improve carbohydrate metabolism and the hemostasis system. Diabeton MR (modified-release gliclazide) not only normalizes carbohydrate metabolism but also improves the prothrombotic state [4]. Compared to other drugs in its class, Diabeton MR enhances the first phase of insulin secretion and acts on the second phase as well. It has a lower risk of causing hypoglycemia and reduces hepatic glucose production [5]. In addition to these properties, Diabeton MR decreases platelet adhesion and aggregation and increases fibrinolysis [4].

Another drug, metformin, significantly reduces the level of the tissue plasminogen activator inhibitor PAI-1 and affects the activation of factor XIII and fibrin polymerization [6]. Gliclazide and glibenclamide are also of interest for their effects on carbohydrate metabolism and the hemostasis system. When comparing the influence of gliclazide and glibenclamide on glycemic control and coagulation, it was shown that both drugs reduced glycated hemoglobin levels after six months of treatment. In the group receiving gliclazide, a reduction in platelet aggregation and an increase in antithrombin III levels were observed, while in the group receiving glibenclamide, platelet aggregation levels remained unchanged and antithrombin III levels even decreased [7, 8].

Medicinal substances of both synthetic and plant origin, as well as drugs long used in medical practice, have the ability to prevent further progression of diabetes mellitus (DM) and the development of its complications. A pressing issue in modern pharmacology remains the search for new antidiabetic drugs with high therapeutic activity and the ability to prevent further development of circulatory disorders.

The aim of this study is to investigate the effects of the polyphenol ANK-1 and the extract ITL-2 on carbohydrate metabolism indicators and the hemostasis system in rats with experimentally induced type 2 diabetes.

Materials and Methods: The study was conducted on 30 sexually mature, non-pedigree white male rats weighing 280–340 g, which were kept in vivarium conditions. The experimental model of type 2 diabetes mellitus was induced under natural light conditions using a complete, nutritionally balanced laboratory animal diet.

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All procedures were performed in accordance with the requirements of the World Society for the Protection of Animals and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (European Convention, 1986).

Type 2 diabetes was induced by feeding the animals a high-fat diet for 60 days, followed by a single intraperitoneal injection of streptozotocin. On the 61st day, after 12 hours of fasting, the rats were injected intraperitoneally with a freshly prepared streptozotocin solution (35 mg/kg in 0.1 M citrate buffer, pH 4.5). After the injection, the animals were given sufficient access to food and water to prevent the development of hypoglycemic coma. Streptozotocin causes acute necrosis of pancreatic beta cells and depletion of insulin from them; significant hyperglycemia typically develops within 24–72 hours and persists for a prolonged period [9].

On the third day after injection, blood samples were collected dropwise from the conjunctiva of the rat's eye into Eppendorf tubes: 50 µL of blood without stabilizer for antithrombotic activity assessment and 0.5 mL of blood in a citrate solution (1:9 ratio), which was then mixed thoroughly and centrifuged (OPN-8 rotor RU180 L8000g) at 3000 rpm for 10 minutes [9]. The following coagulation parameters were examined: prothrombin time (PT), activated partial thromboplastin time (APTT), plasma recalcification time (PRT, seconds), and fibrinogen (F) [9], using a coagulometer (CYANCoag, Belgium, CY003, SN:5400439) with reagent kits from CYPRESS DIAGNOSTICS, Germany.

Biochemical parameters were determined in serum using a semi-automated biochemical analyzer "CYANSmart" with test kits from Cypress Diagnostics, Belgium. The measured indicators included: glucose (mmol/L), total protein (g/dL), alanine and aspartate aminotransferases (ALT, AST) in U/L, total cholesterol (mmol/L), and triglycerides (mmol/L).

The polyphenolic compounds used in this study included ANK-1 (derived from *Pistacia vera*, $C_{48}H_{36}O_{30}$, molecular weight 109) and ITL-2 (an extract from *Isatis tinctoria* leaves), along with a comparison drug, Diaglizid® MR tablets (30 mg), manufactured by "Farmak," Ukraine.

Experimental data were statistically processed. Data analysis and figure preparation were carried out using the Origin 7.1 software (Microsoft, USA). The parametric Student's t-test was used for comparing independent samples that followed a normal distribution. t-values were calculated for a 95% confidence level ($p \leq 0.05$). Arithmetic means and standard errors from 3–5 independent experiments are presented in histograms and tables.

Results and Discussion:

In the rat model of type 2 diabetes mellitus (T2DM), both biochemical and hemostatic changes were observed. By the third day after the single intraperitoneal injection of streptozotocin, a significant increase in glucose levels and other biochemical indicators was noted in the control group, along with signs of hypercoagulation compared to intact animals.

As shown in Table 1, the glucose level in the control group increased 3.5 times ($p = 0.0001$), AST and ALT levels increased 3.2 times ($p \leq 0.01$) and 2.2 times ($p \leq 0.01$), respectively, compared to the intact group (3.1±0.12 mmol/L, 31.5±4.2 and 49.2±2.1 U/L). Additionally, cholesterol levels increased by 17%, and triglyceride levels rose by 27.6% ($p \leq 0.01$).

Table 1. Changes in Blood Biochemical Parameters in Control Rats with Type 2 Diabetes Mellitus (M±m, n=5)

research groups	Total protein, g/L	ALT, U/L	AST, U/L	Glucose, mmol/L	Total cholesterol, mmol/L	Triglycerides, mmol/L
intact	65,4±2,8	31,5±4,2	49,2±2,1	3,1±0,12	1,8±0,08	0,87±0,02
Control on Day 3 with Type 2 Diabetes	64±1,3	97,3±6,8*	64,1±5,2*	10,9±0,8*	2,1±0,12*	1,11±0,1*

*p ≤0,01 in comparison to the intact group of animals

A single subcutaneous injection of streptozotocin led to a significant reduction in prothrombin time (PT), activated partial thromboplastin time (APTT), and plasma recalcification time (PRT), as well as an increase in fibrinogen (FIB) levels compared to intact animals.

Table 2. Changes in Coagulation Parameters in Rats with Type 2 Diabetes Mellitus

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(M±m, n=6)

Experimental Groups	Coagulation Parameters				AA	SCI
	PT, sec	APTT, sec	PRT, sec	FIB, mg/dL		
Intact	22,0±1,4	40,6±3,0	46,4±2,5	325,8±24		
Control on Day 3 with T2DM	12,9±1,15*	21,7±1,9*	24,3±2,3*	625,0±35*	1,46	74,1

*p ≤0,01 in comparison to the intact group of animals

According to the data presented in Table 2, streptozotocin in the control group of animals induces **hypercoagulation**, which is evidenced by a significant reduction in **prothrombin time (PT)** on the third day after streptozotocin administration—by **70.5%** (p = 0.001) compared to baseline values (22.0 ± 1.4 sec). The **activated partial thromboplastin time (APTT)**, which reflects the intrinsic coagulation pathway, also showed significant changes and was the most indicative of a deficiency in factors involved in the intrinsic mechanism of prothrombinase formation. Moreover, the number of **platelets**, **fibrinogen levels**, and their **functional activity** decreased by **87.1%** (p = 0.001) relative to the intact group (46.4 ± 2.5 sec).

The **plasma recalcification time (PRT)** also revealed a **50.4%** decrease (p = 0.0001), indicating a deficiency of factors involved in the intrinsic coagulation pathway, as well as reduced fibrinogen levels, platelet count, and platelet function. At the same time, **fibrinogen concentration increased by 91.2%** (p = 0.0002) in the T2DM control group, reaching **325.8 ± 24 mg/dL**.

The study of the **prophylactic effects of the polyphenol ANK-1 and extract ITL-2**, in comparison with **Diaglizid**, on the **biochemical blood parameters** of rats with type 2 diabetes showed a **statistically significant reduction in glucose concentration by 1.66 times** (p ≤ 0.01), **AST by 1.6 times** (p ≤ 0.01), and **ALT by 3.1 times** (p ≤ 0.01) compared to the control group (10.9 ± 0.8 mmol/L, 31.5 ± 4.2 and 49.2 ± 2.1 U/L, respectively). Changes in the other biochemical parameters were not statistically significant (Table 3).

Table 3. Biochemical Blood Parameters with Prophylactic Administration of ANK-1, ITL-2, and Diaglizid in Rats with Type 2 Diabetes Mellitus (M±m, n=6)

Drugs	Total Protein, g/L	ALT, U/L	ACT, U/л	Glucose, mmol/L	Total Cholesterol, mmol/L	Triglycerides, mmol/L
Intact	65,4±2,8	31,5±4,2	49,2±2,1	3,1±0,12	1,8±0,08	0,87±0,02
T2DM on Day 3	64±1,3	97,3±6,8*	64,1±5,2*	10,9±0,8*	2,1±0,12*	1,11±0,1*
ANK-1	65,0±2,1	41,5±4,2**	48,2±3,8**	6,4±0,4**	1,9±0,11	0,89±0,07
ITL-2	65,4±2,8	46,7±3,2**	40,8±3,2**	7,0±0,57**	1,8±0,08	0,97±0,02
Diaglizid	64,1±1,1	45,2±3,4**	41,6±2,8**	8,0±0,67**	1,7±0,07	0,98±0,02

* p ≤ 0.01 compared to the control group of animals with T2DM 2.

In the study of the prophylactic effects of the polyphenol **ANK-1** and the extract **ITL-2**, compared to the effect of **Diaglizid**, in blood coagulation tests in rats with type 2 diabetes, a normalization of hemostatic parameters was observed (Fig. 1 and Fig. 2).

It is known that the **APTT test** indicates a deficiency or inhibition of one of the following factors: **XII, XI, IX, VIII, X, V, and II** [8]. The **PT test** determines **prothrombin time**, reflecting the presence or activity of prothrombin complex factors (VII, X, V, II) involved in the **extrinsic pathway** of blood coagulation [10–11].

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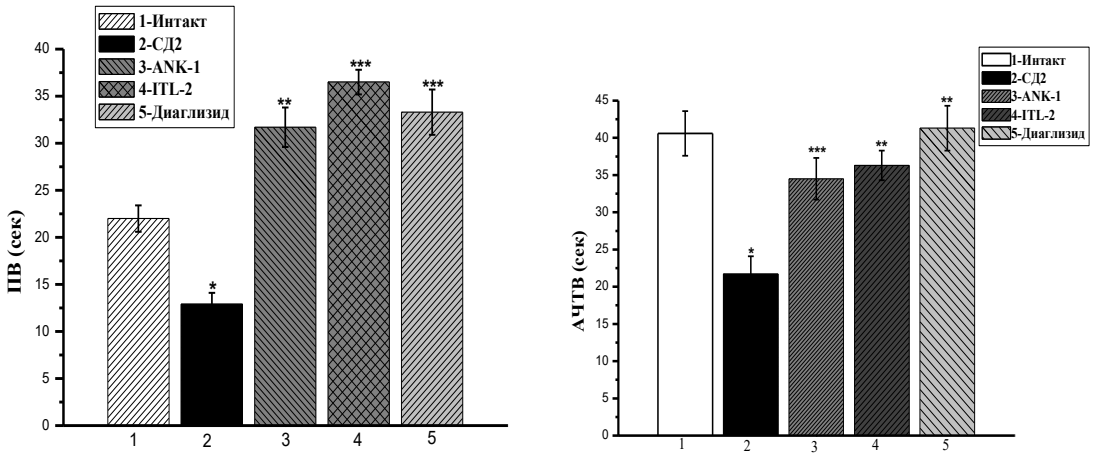

Fig.1. (A)- Study of the effect of polyphenols and the reference drug on prothrombin time (PT). (B) – Study of the effect of polyphenols and the reference drug on APTT.

* — $p < 0.05$; ** — $p < 0.01$; *** — $p < 0.001$. (n = 6).*

Prophylactic administration of the **ANK-1** compound resulted in a **significant increase in PT by 52.0%** ($p = 0.009$) compared to the control (12.9 ± 1.15 sec), **APTT by 84.0%** ($p = 0.001$) compared to control (21.7 ± 1.9 sec), **PRT increased by 207%** ($p = 0.01$) compared to control (33.0 ± 2.3 sec), while **fibrinogen concentration decreased by 74.7%** ($p = 0.01$) (control: 641.0 ± 39 mg/dL).

In the group of animals that received **ITL-2 prophylactically**, a **significant increase in PT by 102.0%** ($p = 0.01$) was observed compared to the control group with T2DM (15.2 ± 1.17 sec), **APTT increased by 76.0%** ($p = 0.001$), **PRT increased by 144.0%** ($p = 0.01$), while **fibrinogen levels decreased by 60.1%** ($p = 0.01$).

In the group treated with the **reference drug Diaglizid**, **PT increased by 163.4%** ($p = 0.01$), **APTT increased by 63.6%** ($p = 0.01$) compared to the control, **PRT increased by 255%** ($p = 0.01$), and **fibrinogen concentration decreased by 30.3%**. % (p=0,01)

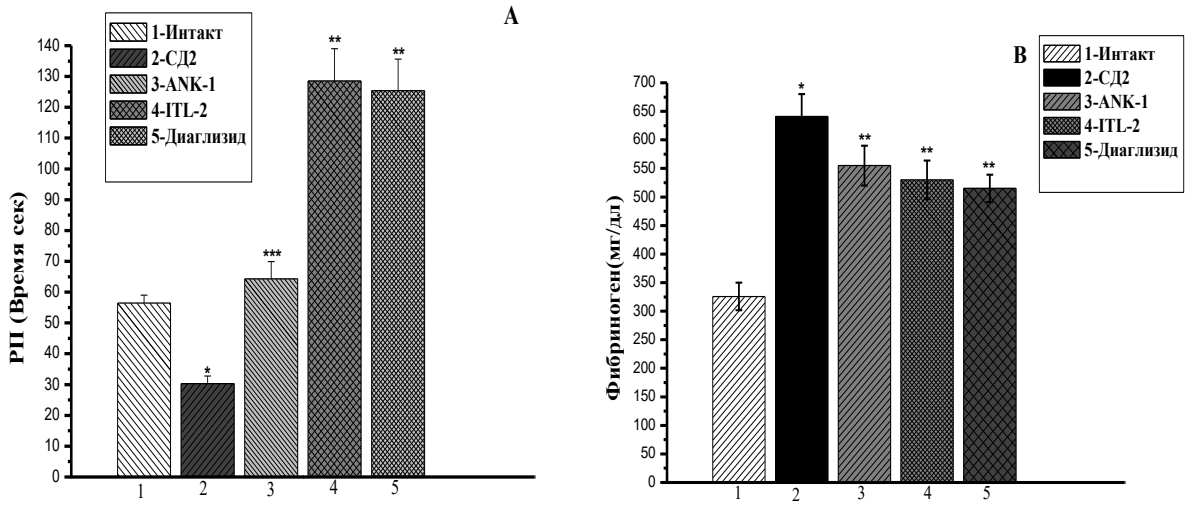

Fig. 2. (A) – Study of the effect of polyphenols and the drug Diaglizid on plasma recalcification time (PRT). (B) – Study of the effect of polyphenols and the drug on the fibrinogen test. * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$. (n = 6)

Thus, PRT and APTT on Day 3 under the influence of the studied compounds and the reference drug did not significantly change compared to the control. Fibrinogen levels in the experimental groups approached baseline values, and only in the group treated with the reference drug exceeded the baseline.

As a result, the conducted research allowed the following conclusions:

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1. In the control group of animals, throughout the experiment, there was a significant increase in biochemical parameters such as AST, ALT, glucose, and the levels of triglycerides and cholesterol.
2. In rats with type 2 diabetes mellitus, hypercoagulation was observed, induced by streptozotocin, through both the extrinsic (PT) and intrinsic (APTT) coagulation pathways, leading to a decrease in PT, APTT, and PRT, and an increase in fibrinogen levels.
3. All tested compounds, when administered orally at a dose of 10 mg/kg, showed a normalizing effect on blood coagulation even with prophylactic administration.
4. The most pronounced antithrombotic effects were observed in the ANK-1 and ITL-2 compounds.

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