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ASSESSMENT OF THE BIOCHEMICAL CORRECTION MECHANISMS OF THE UZBEK FLORA PLANT PLANTAGO LANCEOLATA L. UNDER EXPERIMENTAL DIABETES CONDITIONS

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Abstract
In a laboratory study, the effect of the sum of polyphenols isolated from the plant P. lanceolata on the peroxidation process in rats with experimental alloxan-induced diabetes was studied. According to the results of the study, when the sum of polyphenols isolated from the plant P. lanceolata L., a member of the Plantaginaceae family, was administered at doses of 50 mg/kg and 100 mg/kg, the amount of lipid peroxidation products decreased compared to the control group, and the activity of antioxidant enzymes was higher than in the control group.

Keywords: Plantaginaceae, polyphenol, diabetes. P. lanceolata L., antioxidant, lipid, peroxide oxidation.

Introduction

ОЦЕНКА МЕХАНИЗМОВ БИОХИМИЧЕСКОЙ КОРРЕКЦИИ РАСТЕНИЯ PLANTAGO LANCEOLATA L. ФЛОРЫ УЗБЕКИСТАНА В УСЛОВИЯХ ЭКСПЕРИМЕНТАЛЬНОГО ДИАБЕТА

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Аннотация:
В лабораторном исследовании изучено влияние суммы полифенолов, выделенных из растения P. lanceolata, на процессы перекисного окисления липидов у крыс с экспериментальным аллоксановым диабетом. Согласно результатам исследования, при введении суммы полифенолов, выделенных из растения P. lanceolata L., относящегося к семейству подорожниковых, в дозах 50 и 100 мг/кг, количество продуктов перекисного окисления липидов снижалось по сравнению с контрольной группой, а активность антиоксидантных ферментов была выше, чем в контрольной группе.

Ключевые слова: Plantaginaceae, полифенол, диабет. P. lanceolata L., антиоксидант, липид, перекисное окисление.

INTRODUCTION

Today, the number of cases of diabetes mellitus is increasing worldwide. This has a serious impact on the body, leading to negative consequences such as metabolic disorders and weakened immunity. In particular, this disease also promotes the development of other concomitant diseases. Therefore, the development of prevention and treatment methods based on natural substances is of urgent importance. Based on the above circumstances, this study is aimed at studying natural substances in the pathogenesis of diabetes.

LITERATURE REVIEW

The importance of P. lanceolata L. in medicine and folk medicine: According to the European Medicines Agency [1], substances such as pectic polysaccharides, rhamnogalacturonan, arabino-galactan and α-D-glucan have been isolated from the leaves of P. lanceolata L. [2]. Studies have shown that the structure of polysaccharides mainly consists of arabinose, galactose, rhamnose and galacturonic acids, and the polysaccharides in their composition have been shown to have immunomodulatory, antimicrobial and antioxidant activities [1,3,4].
Plantago lanceolata has been used by North Africans as a medicinal plant for wounds, boils, burns, and inflammation, as a hemorrhoid treatment, and as a fever reducer [5]. It has also been widely used in traditional medicine as a remedy for diarrhea, dysentery, anesthetic, connective tissue repair, anti-inflammatory, anthelmintic, analgesic, antihistamine, rheumatic, and antitumor agent [6,7].

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Shuma Fayera et al. conducted studies on the antimicrobial activity of the leaf extract of Plantago lanceolata by phytochemically examining it. During the studies, the identification of important phytochemicals such as steroids, alkaloids, flavonoids, tannins, saponins, glycosides, phenols, terpenoids in the leaf extract is a sufficient scientific basis for using the plant as a medicinal plant. Pure compounds isolated from the crude extract of the leaves showed strong antibiotic properties when tested against bacteria; E. coli, S. thyphei, S. aureus and S. agalatiae and fungi; A. niger, F. solani [6,7,8].

Antioxidants are an essential component of the complex treatment of diabetes, and it is important to study the effect of natural antioxidants isolated from plants on lipid peroxidation and the course of microangiopathy in patients with diabetes. Flavonoids and their derivatives are heterocyclic compounds, which, due to their antioxidant and membrane-stabilizing properties, exhibit properties that reduce the permeability and fragility of blood vessels. Polyphenolic compounds, interacting with free radicals and forming inactive phenol radicals, sharply slow down the oxidation of lipids in the body [9,10].

During the study, based on the mechanisms of correction of polyphenols, the effects of the sum of polyphenols synthesized from the P. lanceolata plant on the peroxidation process in rats with experimental diabetes were investigated.

MATERIALS AND METHODS

The intensity of lipid peroxidation processes was assessed by the amount of their products - malondialdehyde (MDA) and diene conjugates (DC) in blood serum. The amount of MDA was determined using the method of L.I. Andreeva et al. (1988) [11]. This method is based on the interaction of MDA, which is formed during the peroxidation of unsaturated fatty acids containing 2–3 diene bonds, with thiobarbituric acid. The extinction of the solution was measured at a wavelength of 532 nm using a METTLER TOLEDO UV 5 (Switzerland) spectrophotometer relative to the control. The amount of products reacting with thiobarbituric acid was calculated using the molar extinction coefficient of MDA, which is equal to 1.56·105 mol·cm–1. The amount of MDA was expressed in mmol/l.

The amount of diene conjugates (DC) in blood serum was determined by the method of V.B. Gavrilov and M.I. Mishkorudnaya (1983) [12]. The method is based on the determination of the optical density of DC extracted in an acidic

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medium using a heptane–isopropanol mixture at a wavelength of 233 nm. The optical density was measured relative to the control using a METTLER TOLEDO UV 5 (Switzerland) spectrophotometer. The amount of DC was calculated in mmol/l.

Determination of the activity of antioxidant system enzymes: The activity of the antioxidant system was assessed by the activity of superoxide dismutase (SOD) and catalase (CAT). SOD activity was determined by the method of V.G. Mkhitaryan and G.E. Badalyan (1978) [13]. The method is based on the ability of the enzyme to inhibit the reduction reaction of nitrotetrazolium blue in an alkaline medium. Calculations were made on the percentage of inhibition of the reduction of nitrotetrazolium blue (T%):

$$T\% = \frac{E_к - E_о}{E_л} \cdot 100\%$$

SOD activity was calculated based on the following formula:

A = T % / 100 % – T % · 0.2 · N,

where: A – enzyme activity (IU/ml),

0.2 – amount of serum obtained,

N – degree of dissolution.

Catalase activity (CAT) was determined by the method of M.A. Korolyuk et al. (1988) [14; 16–18–p.]. The method is based on the formation of a strong yellow color of hydrogen peroxide with molybdenum salts. The color intensity was measured at a wavelength of 410 nm using a METTLER TOLEDO UV 5 (Switzerland) spectrophotometer. The enzyme activity was calculated using the following formula:

E = (Ax – Ao) υ · t · K

Here: E – KAT activity, U/ml;

Ax and Ao – extinction of control and experimental samples;

υ – volume of sample introduced (0.1 ml);

t – incubation time (600 s);

K – millimolar extinction coefficient of hydrogen peroxide equal to 22.2 103 mM –1 cm –1.

ANALYSIS AND RESULTS

On days 7, 14 and 21 of experimental diabetes, the amount of lipid peroxidation products - MDA - was 152.9, 159.3 and 43.7% higher than the intact value, and

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the amount of DK - 194.2, 116.3 and 19.2% higher than the intact value. SOD activity in the blood was 35.6, 29.6 and 14.8% lower than the intact value on days 7, 14 and 21 of the disease, respectively. KAT activity was 81.7 and 32.2% higher than the intact value on days 7 and 14 of the disease, and 40.3% lower on day 21. P. lanceolata L. When the plant polyphenols were administered at a dose of 50 mg/kg, the MDA level on days 7, 14, and 21 of the disease was 32.5, 43.7, and 15.1% lower than the control. At the same time, the MDA level was significantly higher on these same days than the intact index by 70.7, 46.0, and 22.1%.

When rats with experimental diabetes were given the total polyphenols of P. lanceolata l. at a dose of 50 mg/kg, the amount of DK on days 7, 14, and 21 of the disease was statistically significantly lower by 27.7, 23.7, and 12.2% compared to the control. At the same time, the amount of DK on days 7 and 14 of the disease was significantly higher by 112.8 and 65.1% compared to the intact value. The amount of DK on day 21 of the disease did not differ significantly from the intact value.

1- Table Effect of P. lanceolata plant polyphenols (at a dose of 50 mg/kg) on lipid peroxidation and antioxidant system activity in experimental diabetes dynamics

Groups		Statistical indicators	LPO products		AOT activity	
			MDA, mmol/l	DK, mmol/l	SOD, sh.b./mg protein	KAT, μKat/mg protein
Intact		M ± m	2,63 ± 0,08	1,72 ± 0,03	1,35 ± 0,02	37,68 ± 1,14
		Max ÷ Min	2,88 ÷ 2,42	1,85 ÷ 1,65	1,41 ÷ 1,25	41,41 ÷ 34,12
MD	7 day	M ± m	6,65 ± 0,43	5,06 ± 0,28	0,87 ± 0,05	68,47 ± 4,02
		Max ÷ Min	8,25 ÷ 5,48	5,69 ÷ 3,87	1,08 ÷ 0,75	81,31 ÷ 55,2
		R	0,001	0,001	0,001	0,001
MD + PF	7 day	M ± m	4,49 ± 0,27	3,66 ± 0,18	0,93 ± 0,06	51,22 ± 2,45
		Max ÷ Min	5,22 ÷ 3,58	4,5 ÷ 3,22	1,12 ÷ 0,76	57,43 ÷ 40,84
		R	0,001	0,001	0,001	0,001
		R1	0,001	0,001	Ie	0,001
MD	14 day	M ± m	6,82 ± 0,52	3,72 ± 0,31	0,95 ± 0,04	49,80 ± 3,79
		Max ÷ Min	8,54 ÷ 5,45	4,56 ÷ 2,78	1,05 ÷ 0,8	65,31 ÷ 40,93
		R	0,001	0,001	0,001	0,01
MD + PF	14 day	M ± m	3,84 ± 0,15	2,84 ± 0,15	1,02 ± 0,04	39,66 ± 1,41
		Max ÷ Min	4,3 ÷ 3,45	3,32 ÷ 2,45	1,13 ÷ 0,85	44,36 ÷ 35,12
		R	0,001	0,001	0,001	Ie
		R1	0,001	0,001	Ie	0,05
MD	21 day	M ± m	3,78 ± 0,22	2,05 ± 0,06	1,15 ± 0,03	22,49 ± 1,62
		Max ÷ Min	4,6 ÷ 2,95	2,22 ÷ 1,83	1,28 ÷ 1,08	27,85 ÷ 17,11
		R	0,001	0,001	0,001	0,001
MD + PF	21 day	M ± m	3,21 ± 0,08	1,80 ± 0,04	1,20 ± 0,03	29,44 ± 1,52
		Max ÷ Min	3,5 ÷ 3	1,97 ÷ 1,68	1,31 ÷ 1,11	34,32 ÷ 25,93
		R	0,001	Ie	0,001	0,05
		R1	0,05	0,05	Ie	0,05

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Note: P – confidence level relative to intact indicator, P1 – confidence level relative to control indicators, QD – diabetes mellitus, PF – polyphenols, ie – not reliable. (n=7-8).

When P. lanceolata L. plant polyphenols were administered to experimental diabetic rats at a dose of 50 mg/kg, the SOD activity was not statistically significant (P > 0.05) higher than the control by 6.9, 7.4 and 4.4%, respectively, on days 7, 14 and 21 of the disease. At the same time, the SOD activity was statistically significant by 31.1, 24.4 and 11.1% compared to the intact value on days 7, 14 and 21 of the experiment.

The activity of CAT was 25.2 and 20.4% lower than the control values on days 7 and 14 of the experiment, respectively. On day 21, on the contrary, this indicator was 30.9% higher than the control. When experimental diabetes was treated with the sum of polyphenols at a dose of 50 mg/kg, the activity of CAT was 35.9% higher than the intact value on day 7 of the experiment, at the level of the intact value on day 14, and 21.9% lower than the intact value on day 21.

2- Table Effect of P. lanceolata L. polyphenols (at a dose of 100 mg/kg) on lipid peroxidation and antioxidant system activity in experimental diabetes mellitus

Groups		Statistical indicators	LPO products		AOT activity	
			MDA, mmol/l	DK, mmol/l	SOD, sh.b./mg protein	KAT, µKat/mg protein
Intact		M ± m	2,63 ± 0,08	1,72 ± 0,03	1,35 ± 0,02	37,68 ± 1,14
		Max ÷ Min	2,88 ÷ 2,42	1,85 ÷ 1,65	1,41 ÷ 1,25	41,41 ÷ 34,12
MD	7 day	M ± m	6,65 ± 0,43	5,06 ± 0,28	0,87 ± 0,05	68,47 ± 4,02
		Max ÷ Min	8,25 ÷ 5,48	5,69 ÷ 3,87	1,08 ÷ 0,75	81,31 ÷ 55,2
		R	0,001	0,001	0,001	0,001
MD + PF	7 day	M ± m	3,99 ± 0,16	2,65 ± 0,09	0,94 ± 0,06	42,94 ± 2,03
		Max ÷ Min	4,63 ÷ 3,58	2,95 ÷ 2,27	1,12 ÷ 0,8	50,8 ÷ 37,42
		R	0,001	0,001	0,001	0,05
		R1	0,001	0,001	Ie	0,001
MD	14 day	M ± m	6,82 ± 0,52	3,72 ± 0,31	0,95 ± 0,04	49,80 ± 3,79
		Max ÷ Min	8,54 ÷ 5,45	4,56 ÷ 2,78	1,05 ÷ 0,8	65,31 ÷ 40,93
		R	0,001	0,001	0,001	0,01
MD + PF	14 day	M ± m	3,50 ± 0,07	1,95 ± 0,15	1,04 ± 0,05	37,35 ± 1,84
		Max ÷ Min	3,72 ÷ 3,28	2,33 ÷ 1,45	1,17 ÷ 0,9	41,33 ÷ 31,12
		R	0,001	Ie	0,001	Ie
		R1	0,001	0,001	Ie	0,001
MD	21 day	M ± m	3,78 ± 0,22	2,05 ± 0,06	1,15 ± 0,03	22,49 ± 1,62
		Max ÷ Min	4,6 ÷ 2,95	2,22 ÷ 1,83	1,28 ÷ 1,08	27,85 ÷ 17,11
		R	0,001	0,001	0,001	0,001
MD + PF	21 day	M ± m	3,04 ± 0,07	1,75 ± 0,05	1,17 ± 0,03	31,96 ± 1,43
		Max ÷ Min	3,21 ÷ 2,75	1,88 ÷ 1,6	1,26 ÷ 1,06	37,11 ÷ 29,02
		R	0,001	Ie	0,001	0,01
		R1	0,001	0,001	Ie	0,01

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Note: P – confidence level relative to intact indicator, P1 – confidence level relative to control indicators, QD – diabetes mellitus, PF – polyphenols, ie – not reliable. (n=7-8).

When rats with experimental diabetes were given the total polyphenols of P. lanceolata L. at a dose of 100 mg/kg, the MDA content on days 7, 14 and 21 of the disease was 40.0, 48.7 and 19.6% lower than the control (Table 2). At the same time, the MDA content on the same days was significantly higher than the intact indicator by 51.7, 33.1 and 15.6%. When rats with experimental diabetes were given the total polyphenols of P. lanceolata L. at a dose of 100 mg/kg, the DK content on days 7, 14 and 21 of the disease was statistically significantly lower than the control by 47.6, 47.6 and 14.6%. At the same time, the amount of DK increased by 54.1% compared to the intact indicator on the 7th day of experimental diabetes treatment.

The observed 13.4% increase in DK levels compared to intact levels on day 14 of treatment was not statistically significant ($P > 0.05$). On day 21 of treatment, DK levels in the treated group were virtually indistinguishable from intact levels. When P. lanceolata L. plant polyphenols were administered to experimental diabetic rats at a dose of 100 mg/kg, the SOD activity was not statistically significantly higher than that of the controls by 8.1 and 9.5% on days 7 and 14 of the disease, respectively ($P > 0.05$). On day 21 of the experiment, the SOD activity did not differ from that of the control group. Despite the fact that the SOD activity in the treated group was more favorable than that in the untreated group, the SOD activity on days 7, 14, and 21 of the experiment was statistically significantly lower by 30.4, 23.0, and 13.3% compared to the intact group.

In rats treated with the sum of polyphenols at a dose of 100 mg/kg, the CAT activity was 37.3 and 25.0% lower than the control values on days 7 and 14 of the experiment, respectively. In the blood of rats with experimental diabetes, the CAT activity was 42.1% higher than the control on day 21 of the experiment, on the contrary, on the contrary, on the control. When experimental diabetes was treated with the sum of polyphenols at a dose of 100 mg/kg, the CAT activity was 14.0% higher than the intact value on day 7 of the experiment, almost did not differ from the intact value on day 14, and was 15.2% lower than the intact value on day 21.

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CONCLUSIONS AND SUGGESTIONS

Thus, the results of the study showed that in experimental alloxan-induced diabetes, lipid peroxidation processes are accelerated and changes in the activity of antioxidant enzymes occur. When rats with experimental alloxan-induced diabetes were given the sum of polyphenols isolated from the plant P. lanceolata L. of the Plantaginaceae family at a dose of 50 mg/kg and 100 mg/kg, they were observed to have a decrease in the amount of lipid peroxidation products compared to the control group and a better activity of antioxidant enzymes compared to the control group. From this, it can be concluded that these substances have antioxidant properties.

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