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Study of parameters of lipid peroxidation and antioxidant protection in rats treated with cadmium nitrate with biocorrection by infusion of *Beta vulgaris* seeds

Recently, interest in natural antioxidants and their use in the food industry has increased. In addition to ensuring maximum safety of the produced product, they also serve as an antioxidant shield for the entire living organism. Plant extracts contain flavonoid compounds that have an antioxidant effect. In this regard, the purpose of the article is to study the antioxidant properties of infusion of *Beta vulgaris*'s seeds, to establish the relationship between acute and subacute inoculation of rats with cadmium nitrate and oxidative stress. For this experimental study, we used 50 male rats. The animals were exposed to Cd nitrate, with an initial weight of 180 ± 30 g. The first groups of rats were injected with cadmium nitrate at a dose of 0.1 g/L inter peritoneal, the second groups received a 0.01% solution of cadmium nitrate 1 ml 5 days a week, orally for 10 and 24 days. The state of oxidative metabolism in the blood was investigated. To assess the state of oxidative metabolism in the blood, the primary, secondary and end products of lipid peroxidation were determined, such as diene conjugates (DC), cetodiene (CD), malondialdehyde (MDA), Schiff bases (SHB), and the activity of glutathione peroxidase (GP), catalase (CAT), and adenosine deaminase (ADA) enzymes were evaluated by the parameters of antioxidant protection. The mean ± SEM values were calculated for each group to determine the significance of the intergroup difference. Each parameter was analyzed separately using the one-way analysis of variance (ANOVA) test. To determine the difference between groups, Student's "t"-test was used. Studies have shown that with the use of cadmium in the blood of experimental animals, there is a violation of catabolits of lipoperoxidation, depending on the length of the days of poisoning, but a significant decrease in the biocorrection of *Beta vulgaris*'s seeds. Because of in this study, we found that the activity levels of antioxidant defense enzymes were significantly increased in the blood of experimental animals, which biocorrected with beetroot seed infusion. In conclusion, our study allows us to state that the use infusion of *Beta vulgaris*'s seeds in subacute and acute experiments with poisoning of experimental rats with cadmium nitrate has an antioxidant protective effect.

Keywords: cadmium nitrate, infusion of *Beta vulgaris*'s seeds, lipid peroxidation, antioxidant protection, biocorrection.

Introduction

Cadmium (Cd), like other heavy metals such as arsenic, lead, and chromium, is a persistent inorganic toxic pollutant that comes mainly from various anthropogenic influences such as industrialization and mining [1]. It is easily absorbed by plant roots due to its relatively high mobility in the soil-plant system and can pose a serious threat to animal and human health when entering the food chain [2]. Its presence in the environment creates a number of problems both for animals at various functional levels and for humans.

Cadmium can cause oxidative damage in various tissues by enhancing membrane lipid peroxidation and altering the antioxidant system of cells. Peroxidative damage to the cell membrane can lead to damage to cellular components due to the interaction of metal ions with cell organelles. Cd increases the production of reactive oxygen species (ROS) and causes oxidative stress. Excessive cellular levels of ROS cause damage to proteins, nucleic acids, lipids, membranes, and organelles.

Oxidative stress is the cause of lipid peroxidation (LPO), in which a whole cascade of sequential free radical reactions occurs with the formation of various chemical compounds (alcohols, aldehydes, ketones) with high biological activity and toxicity [3]. As a result of lipid peroxidation, the structure of cell membranes is disrupted, their plasticity decreases, and their permeability changes [4].

It has been suggested that LPO is involved in most of the toxicity of heavy metal ions, and oxidative damage may be responsible for their toxic effects [5, 6]. Cd induces oxidative damage in various tissues by increasing membrane lipid peroxidation and altering antioxidant enzymes involved in the removal of activated oxygen species [7, 8].

Natural antioxidants are popular drugs that are used by most people and have few side effects. Natural antioxidants play an important role in reducing free radical damage caused by Cd toxicity.

Bioactive compounds commonly observable in fruits, vegetables, herbs, and other plants have possible health benefits such as antioxidant, anti-carcinogenic, atherosclerotic, anti-mutagenic, and angiogenesis inhibitor activities [9, 10].

Beta vulgaris L. is one of the most commonly produced vegetables worldwide, plant extracts containing phenolic compounds have recently been researched to find new natural food ingredients. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which may play an important role in their ability to chelate and neutralize free radicals, quenching singlet and triplet oxygen, and degrade peroxides [11]. In view of the above, we have studied the antioxidant properties of *B. vulgaris*'s seeds infusion during acute and sub-acute inoculation of rats with cadmium nitrate.

Experimental

Preparation of infusion of Beta vulgaris L. seeds

Seeds for research were obtained from the collection of the Michurin agricultural settlement and were identified by the experts of the Department of Botany of the Faculty of Biology and Geography of Karaganda University of the name of academician E.A. Buketov. 3.5 g of dried seeds were taken; they were infused in 200 mL of water at a temperature of 80–90 ºC for 1 hour and filtered. Then the freshly prepared filtrate was used in the study for five hours.

Animals and hemolysate preparation

This research complied with the ethical principles outlined in the European Community Directive (86/609EC) and the requirements of the World Animal Protection (WSPA). In the experiment, a total of 50 male non-linear rats were used. The rats were housed in five per cage and had free access to food and water. They were exposed to a 14–10-h light-dark cycle; the room temperature was controlled at 22±3 ºC. Rats were poisoned with Cd nitrate, which was administered in some animals at a dose of 0.1 g/L single intraperitoneally, other animals obtained 0.01 % 1 mL cadmium nitrate solution 5 days a week orally for 24 days — daily to each animal per orally. Animals were exposed to Cd nitrate when they weighed 180 ± 30 g. Experiments were performed for 10 and 24 days. The 50 non-linear rats were divided into five groups according to:

G1: Rats exposed to Cd (in the form of Cd nitrate), 1 mL per rat single intraperitoneal dose of 0.1 g/L.

G2: This group received Cd nitrate 1 mL per rat single intraperitoneal dose of 0.1 g/L + seeds infusion (3.6 mL per rat during the first 5 hours of the light part of the day) for 10 days.

G3: Rats exposed to Cd (in the form of Cd nitrate), 1 mL per rat for 24 days.

G4: This group received Cd nitrate (1 mL per rat) + seeds infusion (3.6 mL per rat during the first 5 hours of the light part of the day) for 24 days.

G5: Rats (n = 10) received water for 24 days.

Animals were sacrificed by decapitation under ether anesthesia.

Assessment of the state of oxidative stress processes in the blood of laboratory animals is carried out by determining the amount of primary, secondary and end products of lipid peroxidation and antioxidant defenses enzymes.

Diene conjugates (DC) and cetodiene (CD) in erythrocytes were determined according to the unified method of V.N. Ushkalova and G.D. Kadochnikova [12]. Optical radiation of diene conjugates and cetodienes were carried out on a spectrophotometer relative to hexane at wavelengths of 232 and 268 nm.

Malondialdehyde (MDA) in blood plasma was carried out according to the modified method of Y.N. Korobeynikova [13]. The determination of Malondialdehyde was carried out on a spectrophotometer against distilled water at wavelengths of 535 nm and 580 nm.

Determination of the activity of adenosine deaminase (ADA) in the erythrocyte was carried out according to the method of Nemechek I.B. et al. [14]. Activity of ADA was assessed by the rate of decrease of sodium adenosine monophosphate in phosphate buffer. Determination of adenosine deaminase was carried out on a spectrophotometer relative to potassium phosphate buffer at a wavelength of 265 nm.

Determination of the activity of glutathione peroxidase (GPO) in the erythrocyte was carried out according to the method of S.N. Vlasov et al. in the reaction with reduced glutathione [15]. Determination of glutathione peroxidase was carried out on a spectrophotometer at a wavelength of 260 nm relative to water.

Activity catalase (CAT) was carried out according to the method of Korolyuk M.A. et al. [16]. The method is based on the ability of hydrogen peroxide to form a stable colored complex with molybdenum salts. The intensity of the developed color was measured on a spectrophotometer at a wavelength of 410 nm. Optical density is measured at λ 410 nm against control. CAT activity in erythrocytes was expressed in nmol H_2O_2 /mL/min.

Statistics processing

Means ± SEM were calculated for each group to determine the significance of between-group differences. Each parameter was analyzed separately using the one-way analysis of variance (ANOVA) test. Student's "t"-test was used to determine the difference between groups. P <0.05 were considered to be significant.

Results and Discussion

The parameters of lipid peroxidation in the blood of rats poisoned with cadmium nitrate and biocorrection of *B.vulgaris*'s seeds are presented in Table 1.

Table 1

The content of lipid peroxidation products in the blood of rats poisoned with cadmium nitrate and biocorrection of *Beta vulgaris*'s seeds

Parameters	Acute poisoning 1 day, autopsy by day 10 (group 1)	Acute poisoning followed by 10 days of bio correction with infusion of Beta vulgaris seeds (group 2)	24 days of poisoning (group 3)	24 days of poisoning with bio correction with infusion of Beta vulgaris seeds (group 4)	The control (group 5)
DC	0.022 ± 0.006	0.018 ±0.005*	0.016 ± 0.009*	0.011 ± 0.007	0.014 ± 0.001
CD	0.023 ± 0.005	0.021 ± 0.003	0.022 ± 0.001*	0.019 ± 0.001 *	0.021 ± 0.001
MDA	0.268 ± 0.02	0.205 ± 0.16	0.262 ± 0.02 0	0.254 ± 0.007 *	0.265 ± 0.002 *
SHB	0.077 ± 0.017	0.070 ± 0.0 04*	0.044 ± 0.001*	0.029 ± 0.007 *	0.061 ± 0.02
* — significance compared to control P < 0.05					

It is seen from Table 1 that in the first group, the content of lipid peroxidation products increases in the blood plasma of rats in 24 days of cadmium priming compared with the control group. It is worth noting that in group 2, with subsequent refueling with biocorrection of *B.vulgaris*'s seed infusion, the content of lipid peroxidation products decreased compared to the first group. The indicator of diene conjugates and Schiff bases in the second group showed a significantly reduced result compared to the first group by 1.2 and 1.1 times, respectively.

In groups of animals with acute seeding with cadmium nitrate (group 3) and seeding with sub-acute biocorrection (group 4), it was found that the content of cetodienes after biocorrection with infusion of common beet seeds significantly decreased compared with the results of the control and experimental groups.

In the analysis of the secondary LPO products MDA, significant differences in the second group from the values of the first, second and third groups were identified. However, the indicators of the second group by 1.3 times and the fourth group by 1.04 times were lower than the values in comparison with the control group.

In our opinion, changes in the values of the MDA index can be explained by their ability to form intermolecular crosslinks with proteins, turning into a bound shape. As well as changes in MDA during activation of LPO can be explained by the redistribution of secondary metabolites towards other aldehydes.

The above assumption is supported by the increases in the Schiff base indices detected by us when exposed to cadmium nitrate in the blood of rats within 24 days of poisoning cadmium nitrate. At the same time, a significant decrease in the content of Schiff bases in the blood of rats in groups with biocorrection with an infusion of *Beta vulgaris* L. seeds was established.

Adenosine deaminase is not an AOD enzyme; however, metabolic disorders of adenyl nucleotides are accompanied by increased generation of superoxide anions. In our study, the dynamics of changes in the enzyme of adenosine deaminase (ADA) in the blood of rats is presented in Figure 1. The decreases in ADA compared to the control group are shown in the first group. The maximum increase in ADA values was recorded already on the 10th day of the experiment with biocorrection with an infusion of *Beta vulgaris*'s seeds.

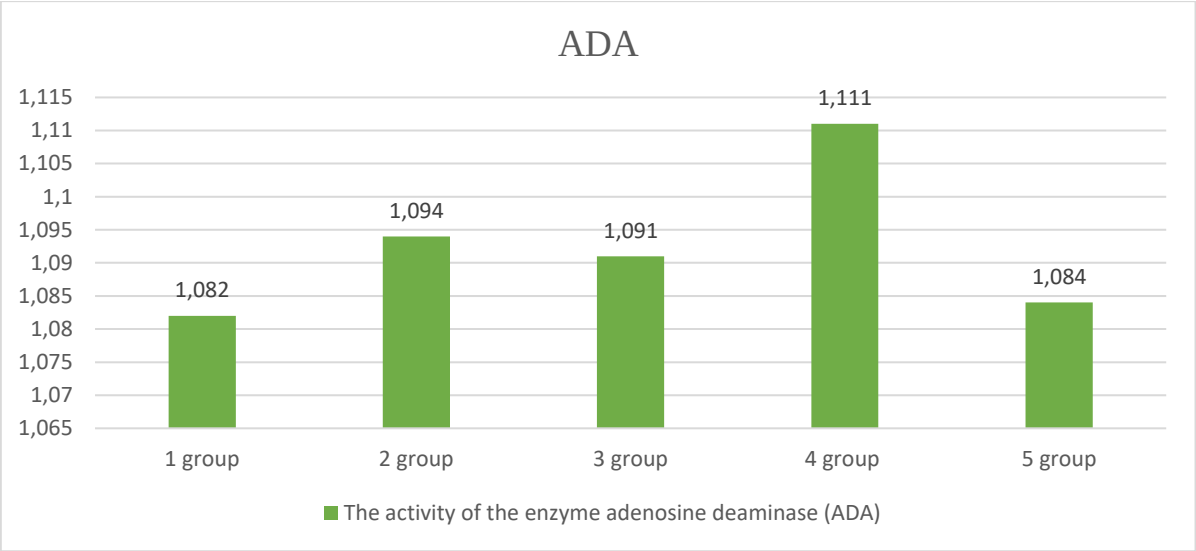

Figure 1. The activity of the enzyme adenosine deaminase (ADA) in the blood of rats poisoned with cadmium nitrate and biocorrection of *Beta vulgaris*‘s seeds

A comparison of the parameters of changes in the activity of antioxidant defense enzymes in rat erythrocytes during subacute and acute poisoning with cadmium nitrate compared with the control group showed a unidirectional tendency to decrease the activity of glutathione peroxidase and catalase. However, a more pronounced increase was observed in the group of acute poisoning with cadmium nitrate, with biocorrection of infusion of *B. vulgaris*’s seeds (group 4), compared with the control group (Tab. 2).

Table 2

The activity of antioxidant defenses enzymes in the blood of rats poisoned with cadmium nitrate and biocorrection of *Beta vulgaris*’s seeds

Parameters	Acute poisoning 1 day, autopsy by day 10 (group 1)	Acute poisoning followed by 10 days of bio correction with infusion of Beta vulgaris seeds (group 2)	24 days of poisoning (group 3)	24 days of poisoning with bio correction with infusion of Beta vulgaris seeds (group 4)	The control (group 5)
GPO	1.198 ± 0.14	1.246 ± 0.29	1.388 ± 0.01*	1.405 ± 0.005 *	1.353 ± 0.09 *
CAT	0.708 ± 0.20	0.826 ± 0.14	0.880 ± 0.45	1.014 ± 0.19	0.982 ± 0.31
* — significance compared to control P < 0.05					

From the data presented in Table 2, it follows that the activity of GPO in rat blood erythrocytes showed a tendency to increase, with biocorrection of infusion of *B. vulgaris*’s seeds with reliable results in group 4. Similar reliable results were shown in group 3 and in the control group.

The results of the experimental study showed that when poisoning cadmium in the blood of experimental animals, a violation of lipoperoxidation catabolits is observed. This observation is consistent with the findings of our study, in which cadmium nitrate increased the products of lipid peroxidation (LPO) in the blood of experimental animals, compared with the control group, but a significant decrease in the biocorrection with infusion of *Beta vulgaris* L. seeds. We assume that when using *Beta vulgaris* L. seeds infusion, the antioxidant molecules interact with active radicals to form low-active radicals. Oxidation slows down, the rate of formation of free radicals decreases, which leads to the obstruction of the chain reactions that are dangerous for the body, which are triggered by free radicals. Since, in this study, we found that the activity levels of antioxidant enzymes such as catalase, glutathione peroxidase, and adenosine deaminase were significantly increased in the blood of experimental animals with biocorrection of the infusion of *Beta vulgaris*’s seeds.

Conclusions

In conclusion, our research allowed asserting that the use of infusion of *Beta vulgaris* L. seeds in subacute and acute experiments with the poisoning of experimental rats with cadmium nitrate has an antioxidant protective effect.

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А.С. Ерубай, А.Е. Конкабаева, Г. Превост, И.С. Калыманов, Д.Б. Окасов

Кадмий нитраты мен уландырылған егеуқұйрықтардың *Beta vulgaris* тұқымының тұнбасымен биокоррекциялаудағы липидтердің асқынтотығуы және антиоксиданттық қорғаныс параметрлерін зерттеу

Соңғы уақытта табиғи антиоксиданттарға және оларды тамақ өнеркәсібінде қолдануға деген қызығушылық артып келеді. Өндірілген өнімнің қауіпсіздігін қамтамасыз етумен қатар, олар бүкіл тірі ағзаның антиоксиданты қалқаны ретінде қызмет етеді. Өсімдік сығындыларының құрамында антиоксиданттық әсері бар флавоноидты қосылыстар бар. Мақаланың мақсаты *Beta vulgaris* тұқымы тұнбасының антиоксиданттық қасиеттерін зерттеу, егеуқұйрықтарды өткір және жедел субстрат кадмий нитратымен егу және тотығу стресі арасындағы байланысты анықтау. Эксперименттік зерттеуге

50 ақ егеуқұйрық (аталығы) алынды. Cd нитратына ұшыраған егеуқұйрықтардың бастапқы салмағы 180±30 г болды. Егеуқұйрықтардың бір тобына кадмий нитраты 0,1 г/л дозада іш астар ішіне енгізілді, екінші тобына 0,01 % кадмий нитратының ерітіндісін 1 мл-ден аптаның 5 күнінде ауыз арқылы 10 және 24 күннің аралығында ішті. Қандағы тотығу метаболизмінің күйін бағалау үшін диен конъюгаты, кетодиен, малон диальдегид, Шифф негіздері сияқты липидтердің асқын тотығуының бастапқы, қайталама және соңғы өнімдерін анықтау жүргізілді және антиоксиданттық қорғаныс параметрлері бойынша глутатион перкосидаза (ГПО), каталаза (КАТ), аденозиндеаминаза (АДА) ферменттерінің белсенділігі бағаланды. Топтар арасындағы айырмашылықтардың маңыздылығын анықтау да әр топ үшін ± SEM орташа мәні есептелді. Әр параметр біржақты дисперсиялық талдау (ANOVA) тестін қолдана отырып бөлек талданды. Топтар арасындағы айырмашылықты анықтау үшін t-критерий Стьюденті қолданылды. Зерттеу нәтижелері көрсеткендей, тәжірибелік жануарлардың қанында кадмий нитратымен уландырылған күндерінің ұзақтығына байланысты липидтердің асқын тотығу катабалитінің бұзылуы байқалды, бірақ *Beta vulgaris* тұқымы тұнбасымен биокоррекциялауда айтарлықтай төмендеді. Осы зерттеуде тәжірибелік жануарлардың қанында *Beta vulgaris* тұқымы тұнбасымен биокоррекциялағанда антиоксиданттық қорғаныс ферменттерінің белсенділік деңгейі едәуір жоғарылағаны анықталған. Қорытындылай келе, жүргізілген зерттеулер кадмий нитратымен тәжірибелік егеуқұйрықтарды өткір және жедел уландырып, *Beta vulgaris* тұқымының тұнбасын қолданғанда антиоксиданттық қорғаныс әсері бар екенін айтуға болады.

Кілт сөздер: кадмий нитраты, *Beta vulgaris* тұқымының тұнбасы, липидтердің асқын тотығуы, антиоксиданттық қорғаныс, биокоррекция.

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Изучение параметров перекисного окисления липидов и антиоксидантной защиты у крыс при затравке нитратом кадмия с биокоррекцией настоем семян *Beta vulgaris*

В последнее время возрос интерес к природным антиоксидантам и их применению в пищевой промышленности. Помимо обеспечения максимальной сохранности производимого продукта они также служат антиоксидантным щитом всего живого организма. В растительных экстрактах содержатся флавоноидные соединения, обладающие антиоксидантным эффектом. В связи с этим целью статьи явилось изучение антиоксидантных свойств настоя семян *Beta vulgaris*, установление взаимосвязь между острой и подострой затравке крыс нитритом кадмия и окислительным стрессом. Для экспериментального исследования были использованы 50 беспородных белых крыс (самцы). Животные подвергались воздействию нитрата Cd, когда они весили 180 ± 30 г. Одним группам крыс вводили нитрат кадмия в дозе 0,1 г/л внутрибрюшинно, вторые группы получали 0,01 % раствор нитрата кадмия по 1 мл 5 дней в неделю через рот в течение 10 и 24 дней. Было исследовано состояние окислительного метаболизма в крови. Для оценки состояния окислительного метаболизма в крови проводились определение первичных, вторичных и конечных продуктов перекисного окисления липидов, таких как дьеновый коньюгат, кетодиен, малоновый диальдегид, основы Шиффа, и по параметрам антиоксидантной защиты оценивали активность ферментов глутатионперкосидазы (ГПО), каталазы (КАТ), аденозиндезаминазы (АДА). Для каждой группы рассчитывались средние значения ± SEM, чтобы определить значимость межгрупповых различий. Каждый параметр анализировали отдельно с использованием теста одностороннего дисперсионного анализа (ANOVA). Для определения разницы между группами использовался t-критерий Стьюдента. Исследования показали, что при употреблении кадмия в крови у экспериментальных животных наблюдается нарушение катабалитов липоперекисного окисления в зависимости от продолжительности дней затравки, но значительное снижение при биокоррекции настоем семян *Beta vulgaris*. Так как в этом исследовании мы обнаружили, что уровни активности ферментов антиоксидантной защиты были значительно увеличены в крови у экспериментальных животных, с биокоррекцией настоем семян *Beta vulgaris*. Результаты проведенного нами исследования позволяют утверждать, что применение настоя семян *Beta vulgaris* в подострых и острых экспериментах с затравкой экспериментальных крыс нитритом кадмия имеет антиоксидантное защитное действие.

Ключевые слова: нитрат кадмия, настой семян *Beta vulgaris*, перекисное окисление липидов, антиоксидантная защита, биокоррекция.

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