UDC 616.831:612.08:54:599.323.4 doi: https://doi.org/10.15407/ubj97.02.077

CHROMIUM PICOLINATE PREVENTS THE DEVELOPMENT OF OXIDATIVE-NITROSATIVE STRESS AND RESTORES ENDOGENOUS H_2S PRODUCTION IN THE RAT BRAIN UNDER ROTENONE-INDUCED TOXICITY

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Received: 19 December 2024; **Revised:** 03 March 2025; **Accepted:** 25 April 2025

Energy deficit, mitochondrial dysfunction and oxidative stress induced by rotenone may play a decisive role in the pathogenesis of neurodegenerative disorders. Chromium picolinate has shown neuroprotective activity and efficacy in the treatment of Alzheimer's disease The effect of chromium picolinate on the brain under the conditions of rotenone influence has not been studied, and such data could shed light on the pathogenesis of neurodegenerative diseases. The aim of the study was to determine the effect of chromium picolinate on the indices of oxidative-nitrosative stress and the content of sulfide anion and sulfites in the brain homogenate under rotenone administration to rats. Experiments were performed on 24 white, sexually mature male Wistar rats. The animals were divided into 4 groups: control group; chromium picolinate group; rotenone group; group of combined exposure to chromium picolinate and rotenone. Chromium picolinate was administered orally at a dose of 80 μg/kg per day for 21 days. Rotenon was injected subcutaneously at a dose of 1.5 mg/kg every other day. The introduction of rotenone into the body of rats was accompanied by the development of oxidative-nitrosative stress mainly due to the increased activity of NO-synthase inducible isoform, and by the decrease in the content of H_2S and SO_3^{2-} in brain tissue. Oral administration of chromium picolinate against the background of rotenone administration prevents the development of oxidative-nitrosative stress in brain tissue by reducing the production of reactive oxygen and nitrogen forms, promotes the restoration of arginase activity and increases the content of H_2S and SO_3^{2-}.

K e y w o r d s: chromium picolinate, rotenon, oxidative-nitrosative stress, NO-synthase, H_2S, rat brain.

Rotenone is widely used as a selective insecticide in agriculture and in the form of an emulsified rotenone concentrate in freshwater fisheries before stocking with fish, which is of significant economic interest [2]. However, rotenone, as an industrial and environmental toxin, leads to neurodegenerative changes when it enters the human body. Rotenone is known to induce inflammation and oxidative stress [1]. Since rotenone is a lipophilic molecule, it easily overcomes the blood-brain barrier and, entering the mitochondria, inhibits the complex I of the electron transport chain, which leads to a decrease in the formation of ATP, an increase in the production of reactive oxygen species (ROS) and the development of oxidative stress [3]. For neurons, the pathway of oxidative phosphorylation is the main energy supply. Therefore, energy deficit and mitochondrial dysfunction resulting from the development of oxidative stress under the influence of rotenone play a decisive role in the pathogenesis of neurodegenerative disorders. An increase in free radicals, such as reactive oxygen species (ROS) or reactive nitrogen species, causes DNA damage, lipid peroxidation, and protein oxidation [4]. In addition, rotenone is known to induce the formation of α-synuclein-positive cytoplasmic inclusions in nigral neurons similar to Lewy bodies, which can be seen in progressive neurodegeneration in people with Parkinson's disease [5]. Components of the ubiquitin-proteasome system and chaperones have been identi-

fied in Lewy bodies, indicating a failed attempt by the ubiquitin-proteasome system to destroy protein aggregates [6]. Thus, this molecular mechanism also plays an important role in the development of the neurodegenerative process and the death of dopaminergic neurons when rotenone is administered to the body.

It is known that the brain synthesizes endogenous hydrogen sulfide, which has an important effect, in particular, on synaptic transmission and the process of forming new neural connections. Hydrogen sulfide (H_2S) likely acts as a neuromodulator and/or as an intracellular messenger and plays an important role in synaptic remodeling [7, 9]. H_2S, synthesized in the brain, affects the growth and development of neurons and their protection from damage [8].

Hydrogen sulfide stimulates N-methyl-d-aspartate receptors to enhance long-term potentiation, suggesting a novel neuromodulatory role *in vivo*. Activation of N-methyl-d-aspartate receptors leads to intracellular nitration of tyrosine by peroxynitrite. According to Whiteman M. et al., H_2S significantly inhibits peroxynitrite-mediated tyrosine nitration. H_2S can also prevent peroxynitrite-induced cytotoxicity, intracellular nitration, and protein oxidation in SH-SY5Y human neuroblastoma cells [10]. Given that extracellular glutathione levels are very low in the brain, and H_2S has the potential to act as an inhibitor of peroxynitrite-mediated processes *in vivo*, hydrogen sulfide as an antioxidant and scavenger of peroxynitrite may need to be more carefully considered.

In nature, chromium exists mainly in two valence states: hexavalent chromium (VI) and trivalent chromium (III). Chromium (VI) is a toxin, mutagen, and carcinogen commonly used in industrial chrome plating, welding, painting, metal finishing, steel, alloy, cast iron, and woodworking. The cytotoxicity of chromium (VI) is not yet fully understood, but there is evidence that it induces oxidative stress, DNA damage, cell apoptosis and changes in gene expression. In contrast to chromium (VI), chromium (III) is necessary for the proper functioning of insulin, the metabolism of proteins, fats and carbohydrates, and is also recognized as a food additive [11, 17]. Chromium picolinate in the brain of rats modeled with streptozotocin diabetes had a protective role; it activated the antioxidant defense of the brain through the Nrf2 pathway, reducing inflammation through inhibition of NF-κB p65 [12]. Recent studies demonstrate the antidepressant effect of anti-inflammatory drugs [13]. There is evidence that Cr^{3+} may have antidepressant properties, possibly by enhancing the function of monoamines through its ability to increase the transport of amino acids into the brain. In the studies of Franklin M. et al., it was found that the administration of Cr^{3+} picolinate to rats can alter serotonin function in the brain, possibly by altering the sensitivity of central 5-HT2A receptors and enhancing serotonergic and noradrenergic function [14]. Chromium picolinate has shown efficacy in the treatment of Alzheimer's disease by ameliorating cognitive deficits in sporadic dementia by reducing neuroinflammation through effects on the IRS-1/PI3K/AKT/GSK-3β pathway. Its effectiveness has been shown to improve memory, reduce oxidative stress, mitochondrial dysfunction, and up-regulate insulin signaling [15]. The effect of chromium picolinate may also be a consequence of the impact of picolinic acid on the central nervous system. There is evidence that picolinic acid analogs cause profound changes in the metabolism of serotonin, dopamine and norepinephrine in the brain [16]. However, the effect of chromium picolinate under the conditions of action of rotenone has not been studied, and such data could shed light on the pathogenesis of Parkinson's disease and other neurodegenerative diseases.

The aim of the study was to determine the effect of chromium picolinate on the development of oxidative-nitrosative stress and the content of sulfide anion and sulfites in the brains of rats under the conditions of rotenone administration.

Materials and Methods

Experiments were performed on 24 white, sexually mature male Wistar rats weighing 230-320 g. During the entire experiment, the animals were kept in a vivarium in accordance with the rules of zoohygiene and at maintaining a 12/12 light-dark daily cycle with constant aeration at 26°C and humidity (43±2)%. Rats received ad libitum a mixed grain-vegetable diet and water.

The animals were divided into 4 groups: the first group – was the control group (n = 6); Group II (Cr (pic)) – rats (n = 6 that were injected with chromium picolinate according to its administration protocol; Group III (Rotenone) – rats (n = 6), which were administered rotenone according to its administration protocol; Group IV – rats (n = 6), which were simulated chronic rotenone intoxication according to the rotenone administration protocol with the simultaneous administration of chromium picoli-

nate according to the chromium picolinate administration protocol.

Protocol for the introduction of chromium picolinate. Chromium picolinate (Sigma-Aldrich, USA) was administered orally to rats at a dose of 80 μg/kg daily for 21 days [18].

Rotenone administration protocol. Rotenone (Sigma-Aldrich, Germany) received 11 subcutaneous injections of rotenone (1.5 mg/kg; 0.2 ml/kg) dissolved in 1% DMSO every other day [19].

The control group included animals subjected to similar manipulations throughout the study period but injected with a physiological solution. The conditions for keeping animals in the vivarium were standard. Animals were removed from the experiment on the 21st day by bloodletting under thiopental anesthesia.

During the experiments, the recommendations of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986) were followed in accordance with the General Principles of Animal Experiments approved by the 1st National Congress on Bioethics and the requirements of the Procedure for conducting scientific institutions of experiments, experiments on animals (2012). All manipulations with laboratory animals were approved by the Bioethical committee of Poltava State Medical University (Protocol No 228 of 20.06.2024).

The object of research was the frontal lobes of the brain of rats. 10% homogenate was used for biochemical studies. After removing the frontal lobes of the rat brain, they were placed in a Petri dish and minced. The tissue was homogenized in 10 mM Tris (2-amino-2-hydroxymethyl-propane-1,3-diol)-HCl buffer, with a pH of 7.4 (1 g of tissue per 9 ml of medium) for (30-40) seconds. The supernatant liquid was used for biochemical studies. All manipulations were carried out at a temperature from 0° to +4°C (in an ice bath).

Biochemical research methods. Catalase activity was determined by the color of the products formed as a result of the reaction of hydrogen peroxide with ammonium molybdate. The calculation was carried out by the amount of hydrogen peroxide that was catabolized in the presence of 0.1 ml of 10% brain tissue homogenate containing catalase per unit of time [20].

To determine the activity of SOD, the autoxidation reaction of adrenaline in an alkaline environment with the formation of superoxide was used, by comparing the rate of autoxidation of adrenaline in the presence of 0.1 ml of 10% brain tissue homogenate and without it. The activity of SOD was calculated in conditional units (c.u., 1 unit indicates a 50% decrease in the reaction rate) [21].

The production of the superoxide anion radical was determined by the reaction of the latter with nitroblue tetrazolium dihydrochloride (NTDC), which leads to the conversion of NTDC, yellow, to diformazan, blue. The absorption maximum of diformazan in chloroform at a wavelength of 500-570 nm [22].

Free malondialdehyde was evaluated by the specific reaction with 1-methyl-2-phenyl-indole in a mixture of methanol and acetonitrile with the formation of a chromogen (carbocyanine dye) with maximum light absorption at a wavelength of 586 nm [23].

The sulfide anion content was calculated as the H_2S content. H_2S specifically reacts with N-N-dimethyl-para-phenylenediamine in the presence of Fe^{3+} ions and an excess of hydrochloric acid with the formation of a red-pink chromogen with a maximum absorption of light at a wavelength of 667 nm [24].

The content of sulfites (SO_3^{2-}) was determined by the reaction of the interaction of SO_3^{2-} with o-phthalaldehyde in the presence of ammonium chloride at a wavelength of 667 nm [25].

The content of nitrites was determined by determining the diazo compounds formed in the reaction with sulfanilic acid, and then the reaction was carried out with α-naphthylamine (Griess-Ilosvay reagent). As a result, red derivatives (azo dyes) are formed. The color intensity is proportional to the nitrite content [26].

The total activity of NO-synthase (gNOS) was determined by the increase in nitrites after incubation of 10% brain tissue homogenate (0.2 ml) for 30 min in the incubation solution (2.5 ml of 0.1 M Tris buffer, 0.3 ml of 320 mM aqueous of L-arginine solution and 0.1 ml of 1 mM NADPH + H^+ solution). To determine the activity of constitutive isoforms of NO-synthase (cNOS), a 1% solution of aminoguanidine hydrochloride was used and the incubation time was extended to 60 min. The activity of the inducible isoform of NO-synthase (iNOS) was calculated according to the formula: iNOS=gNOS-cNOS [26].

Nitrite and nitrate reductase activity was measured by the decrease in nitrate and nitrite content after incubation of 0.2 ml of 10% brain tissue homogenate in the presence of 0.1 ml of 1% electron donor solution (NADH + H^+) [26].

The content of peroxynitrite of alkali and alkaline earth metals was measured by its reaction with potassium iodide at pH 7.0 in 0.2 M phosphate buffer at the same pH, which gives the product I_3 with a maximum absorption at a wavelength of 355 nm [20].

The content of nitrosothiols was determined by the difference in the content of nitrites (NO_2^-) using the Griess reagent (with Ilosvay's modification) before and after the oxidation of nitrosothiol complexes (S-NO) to nitrites with a mercury chloride ($HgCl_2$) solution [27].

The activity of arginases was determined by the increase in L-ornithine content after incubation of 0.2 ml of 10% tissue homogenate with 0.3 ml of 24 mM L-arginine solution [26].

Determination of the content of 3-nitrotyrosine (3-NT) is carried out after the reduction of 3-NT to the amino group (3-aminotyrosine) and with the subsequent formation of an azo bond with sodium nitrite, which in the reaction with β-naphthol forms a red complex [28].

Mathematical and statistical research methods. Statistical processing of the results of biochemical studies was carried out using a pairwise comparison using the non-parametric Mann-Whitney method. All statistical calculations were performed in the Microsoft Office Excel program and its extension, Real Statistics 2019. The difference was considered statistically significant at $P < 0.05$.

Results and Discussion

We found that the catalase activity in the brains of rats under the conditions of administration of rotenone decreased 3 times compared to the control and 2.5 times compared to the group of animals that were administered chromium picolinate (Fig. 1, *A*) ($P < 0.05$). Under the conditions of administration of chromium picolinate against the background of rotenone intoxication simulation, we noted a 2.5-fold increase in catalase activity compared to the group of animals administered rotenone ($P < 0.05$). The activity of SOD in the brain of rats decreased by 1.45 times in the group of animals that were simulated rotenone intoxication and by 1.19 times in the group of rotenone intoxication corrected with chromium picolinate compared to the control (Fig. 1, *B*) ($P < 0.05$). Compared with the group of animals injected with chromium picolinate, the activity of SOD in the brain of rats decreased by 1.41 times in the group of rotenone intoxication and by 1.16 times in the group of correction of rotenone intoxication with chromium picolinate ($P < 0.05$). The introduction of chromium picolinate against the background of rotenone intoxication leads to an increase in SOD in the brain of rats by 1.22 times compared to the rotenone administration group ($P < 0.05$).

The production of the superoxide anion radical in the brain of rats in all studied groups increased statistically significantly compared to the control: in the group of animals injected with chromium picolinate by 1.06 times, in the group of animals injected with rotenone by 1.48 times and in the group correction of rotenone intoxication with chromium picolinate by 1.24 times (Fig. 1, *C*) ($P < 0.05$). Under the conditions of administration of rotenone, the production of superoxide anion radical in the brain of rats increased by 1.4 times, and in the group corrected with chromium picolinate rotenone intoxication by 1.17 times compared to the group of animals that were administered chromium picolinate ($P < 0.05$). Correction of changes caused by rotenone with chromium picolinate led to a decrease in the production of superoxide anion radical in the brain of rats by 1.2 times compared to the group of rotenone intoxication ($P < 0.05$). The content of malondialdehyde in the brain of rats increased in the group of rotenone intoxication by 1.7 times compared to the control and by 1.9 times compared to the group of animals that were injected with chromium picolinate (Fig. 1, *D*) ($P < 0.05$). In the group of correction of rotenone intoxication with chromium picolinate in the brain of rats, malondialdehyde increased by 1.25 times compared to the control group, and compared to the group of chromium picolinate administration by 1.39 times relatively to the group of animals that were injected with rotenone ($P < 0.05$). However, chromium picolinate under the conditions of rotenone intoxication in the brain of rats reduced the content of malondialdehyde by 1.36 times compared to the rotenone group ($P < 0.05$).

Thus, the introduction of chromium picolinate against the background of rotenone intoxication increased antioxidant protection, significantly reducing oxidative damage to lipids of neuron membranes in the brain of rats.

The content of sulfide anion in the brain of rats decreased by 1.11 times under the conditions of chromium picolinate administration, and by 1.31 times under the conditions of rotenone administration compared to the control (Fig. 2, *A*) ($P < 0.05$). In the group of chromium picolinate administration against

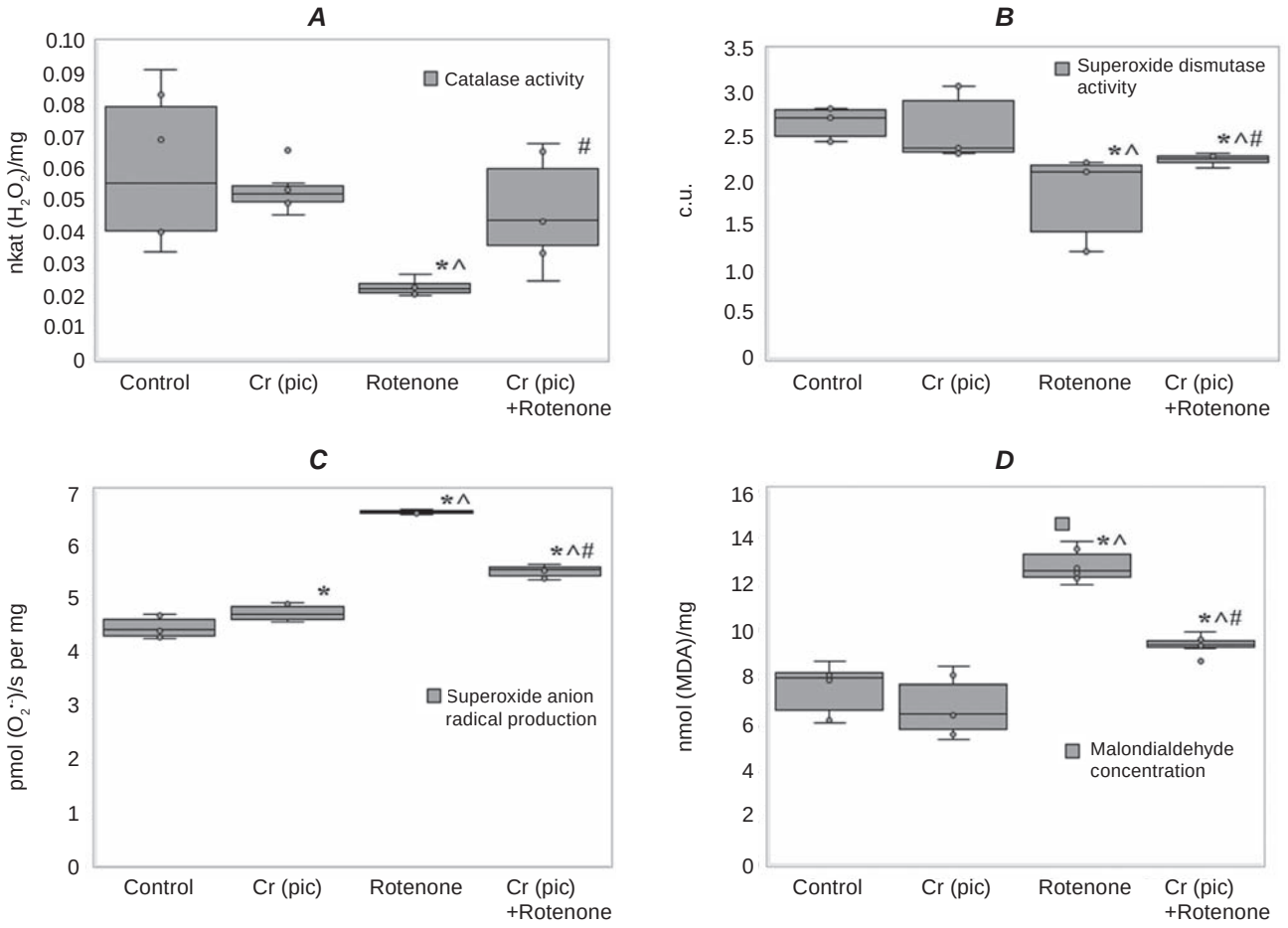

Fig. 1. Prooxidant-antioxidant balance in the brain of rats under the conditions of correction of rotenone intoxication with chromium picolinate. ***A*** *– catalase activity,* ***B*** *– superoxide dismutase activity,* ***C*** *– superoxide anion radical production,* ***D*** *– the content of malondialdehyde. Notes: *P < 0.05 compared to the control group; ^P < 0.05 compared to the group administered chromium picolinate; #compared to the rotenone intoxication group. Symbols: Cr (pic) – chromium picolinate*

the background of rotenone intoxication, the content of sulfide anion in the brain increased by 1.28 times compared to the group of rats administered rotenone ($P < 0.05$). The content of sulfite anion in the brain of rats in the group of rotenone intoxication decreased by 1.15 times compared to the control group and by 1.23 times compared to the group of animals injected with chromium picolinate (Fig. 2, *B*) ($P < 0.05$). Under the conditions of administration of chromium picolinate against the background of rotenone intoxication, the content of sulfite anion increased by 1.2 times compared to the control, by 1.13 times compared to the group of animals that were administered chromium picolinate, and by 1.38 times compared to rotenone group ($P < 0.05$).

Thus, rotenone and chromium picolinate reduce the endogenous formation of sulfide anion in the brain of rats, but their combined effect restores the content of sulfide anion to the level of the control group. Rotenone reduces the content of sulfite anion in the brain of rats, and the use of chromium picolinate as a corrector of rotenone intoxication restores and increases the content of sulfite anion in the brain of rats.

The total activity of NO-synthases in the brain of rats injected with rotenone increased by 1.49 times compared to the control and by 1.53 times compared to the group of animals injected with chromium picolinate (Fig. 3, *A*) ($P < 0.05$). Administration of chromium picolinate against the background of rotenone intoxication reduces the total activity of NO-synthase in the brain of rats by 1.43 times compared to the group of animals that were administered rotenone ($P < 0.05$).

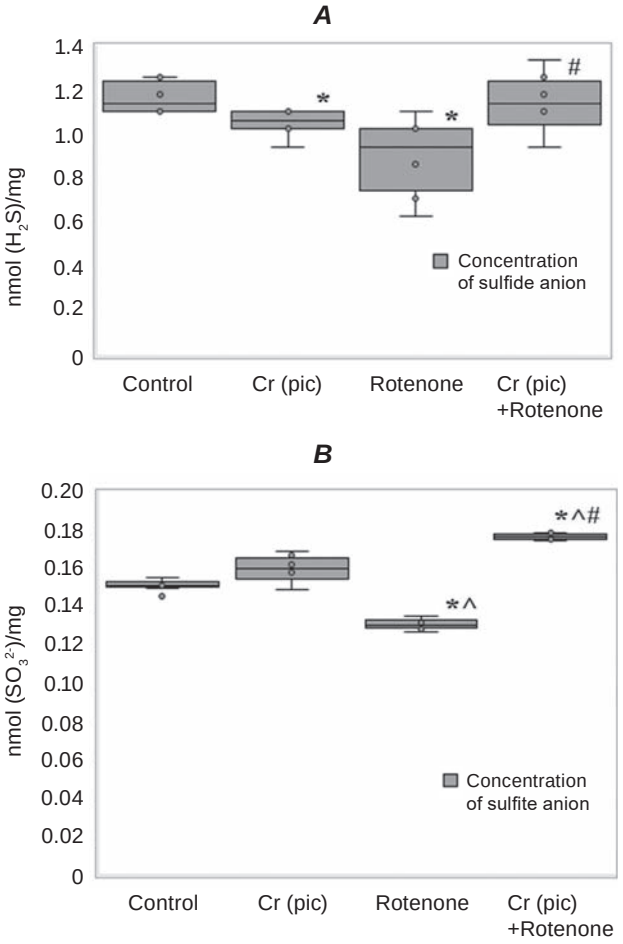

Fig. 2. Contents of sulfide and sulfite anions in the brain of rats under the conditions of correction of rotenone intoxication with chromium picolinate. ***A*** *– the content of sulfide anion,* ***B*** *– the content of sulfite anion Notes: *P < 0.05 compared to the control group; ^P < 0.05 compared to the group administered chromium picolinate; #compared to the rotenone intoxication group. Symbols: Cr (pic) – chromium picolinate*

The activity of iNOS in the brain of rats injected with rotenone increases by 1.5 times compared to the control group of animals and by 1.54 times compared to the group of animals injected with chromium picolinate (Fig. 3, *B*) ($P < 0.05$). The administration of chromium picolinate against the background of rotenone intoxication reduces the activity of iNOS in the brain of rats by 1.44 times compared to the group of animals that were administered rotenone ($P < 0.05$). cNOS activity in the brain of rats injected with chromium picolinate increased 1.05 times compared to the control (Fig. 3, *C*) ($P < 0.05$).

The activity of nitrite reductase in the brain of rats after administration of chromium picolinate increased 1.94 times compared to the control (Fig. 3, *D*) ($P < 0.05$). In the group of rotenone intoxication, the activity of nitrite reductase in the brain of rats decreased by 2.06 times compared to the control and by 4 times compared to the group of animals that were injected with chromium picolinate ($P < 0.05$). Under the conditions of correction of rotenone intoxication with chromium picolinate, the activity of nitrite reductase in the brain of rats increased 1.26 times compared to the control, 2.6 times compared to the group of animals administered rotenone and was reduced 1.54 times compared to the group rats administered chromium picolinate ($P < 0.05$).

The activity of nitrate reductases in the brain of rats decreased by 2.44 times after administration of chromium picolinate, after administration of rotenone by 2.02 times, and after the combination of administration of chromium picolinate and rotenone by 1.24 times compared to the control group of animals (Fig. 3, *E*) ($P < 0.05$). In the group of animals that were injected with chromium picolinate against the background of rotenone intoxication, nitrate reductase activity in the brain of rats increased 1.96 times compared to the group of rats that were injected with chromium picolinate and 1.63 times compared to the group of rotenone intoxication ($P < 0.05$).

The activity of arginases in the brain of rats after the introduction of chromium picolinate increased 1.15 times compared to the control (Fig. 3, *F*) ($P < 0.05$). In the group of rotenone intoxication, the activity of arginase in the brain of rats decreased by 1.5 times compared to the control and by 1.73 times compared to the group of animals that were injected with chromium picolinate ($P < 0.05$). Under the conditions of correction of rotenone intoxication by chromium picolinate, the activity of arginase in the brain of rats decreased by 1.18 times compared to the group of animals that were injected with chromium picolinate and increased by 1.47 times compared to the group of rats that were injected with rotenone ($P < 0.05$).

The content of peroxynitrite in the brain of rats increased in the group of rotenone intoxication by 1.18 times compared to the control and by 1.43 times compared to the animals that were injected with chromium picolinate (Fig. 4, *A*) ($P < 0.05$). Under the conditions of correction of rotenone intoxication with chromium picolinate, the activity of nitrate reductase in the brain of rats increased 1.23

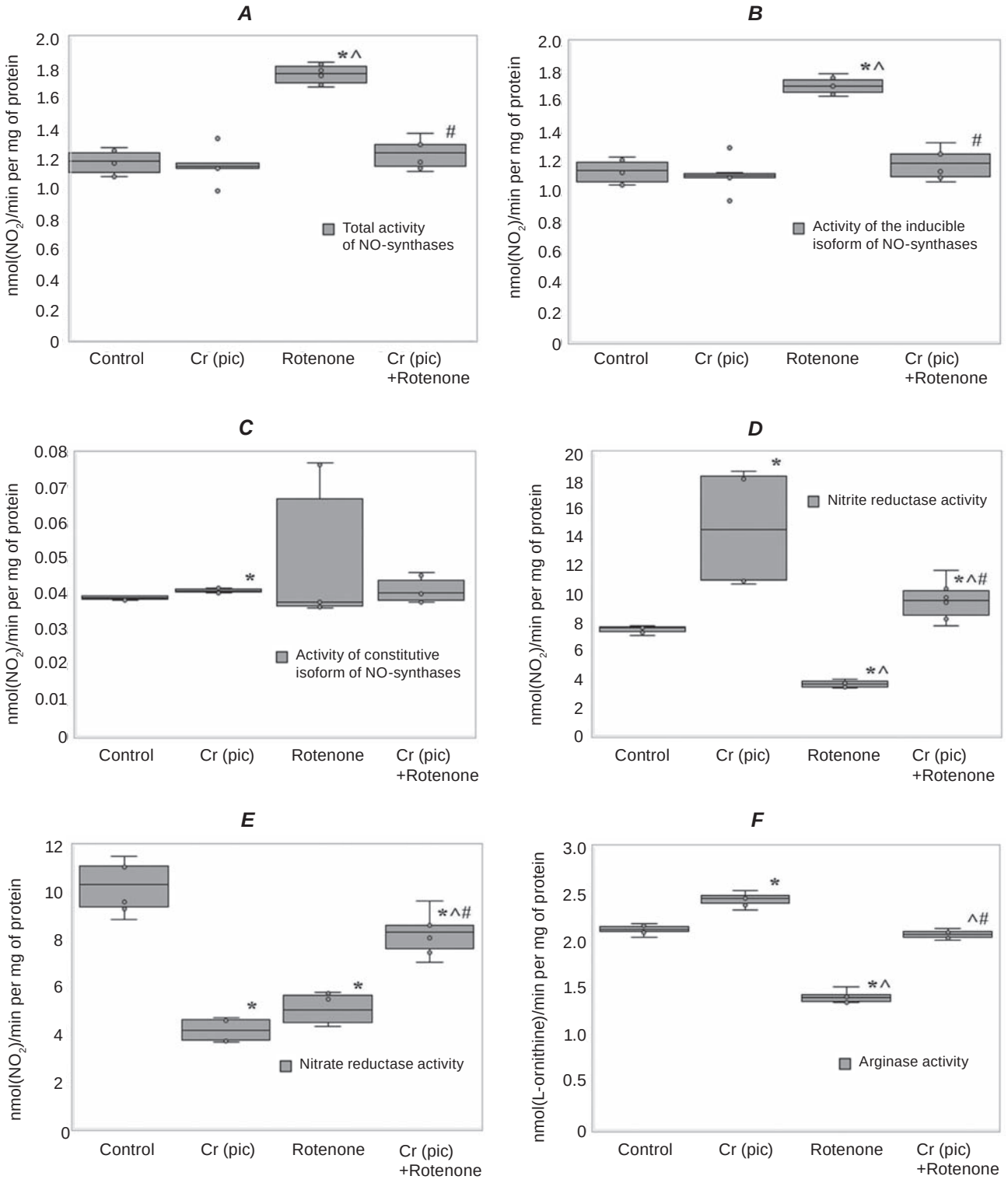

Fig. 3. Activities of enzymes of the nitric oxide cycle in the brain of rats under the conditions of correction of rotenone intoxication with chromium picolinate. ***A*** *– total activity of NO-synthases,* ***B*** *– activity of the inducible isoform of NO-synthases,* ***C*** *– activity of constitutive isoform of NO-synthases,* ***D*** *– nitrite reductase activity,* ***E*** *– nitrate reductase activity,* ***F*** *– arginase activity. Notes: *P < 0.05 compared to the control group; ^ P < 0.05 compared to the group administered chromium picolinate; #compared to the rotenone intoxication group. Symbols: Cr (pic) – chromium picolinate*

times compared to the group of rotenone intoxication ($P < 0.05$).

The content of nitrosothiols in the brain of rats in the group of rotenone intoxication decreased by 3.08 times compared to the control and by 2.92 times compared to the group of animals that were injected with chromium picolinate (Fig. 4, *B*) ($P < 0.05$). Under the conditions of chromium picolinate correction of rotenone intoxication, the content of nitrosothiols increased by 2.08 times compared to the group of rotenone intoxication ($P < 0.05$).

The content of nitrites in the group of animals injected with chromium picolinate increased 1.16 times in the brain of rats compared to the control (Fig. 4, *C*) ($P < 0.05$). In the group of animals that were injected with chromium picolinate against the background of rotenone intoxication, the content of nitrites in the brain of rats decreased by 1.76 times compared to the control, by 2.05 times compared to the group of rats that were injected with chromium picolinate and by 1.9 times compared to rotenone intoxication group ($P < 0.05$).

The content of 3-nitrotyrosine in the brain of rats under conditions of rotenone intoxication increased by 3.34 times compared to the control and by 3.51 times compared to the group of animals that were injected with chromium picolinate (Fig. 4, *D*) ($P < 0.05$). Under the conditions of correction of rotenone intoxication with chromium picolinate, the content of 3-nitrotyrosine in the brain of rats increased

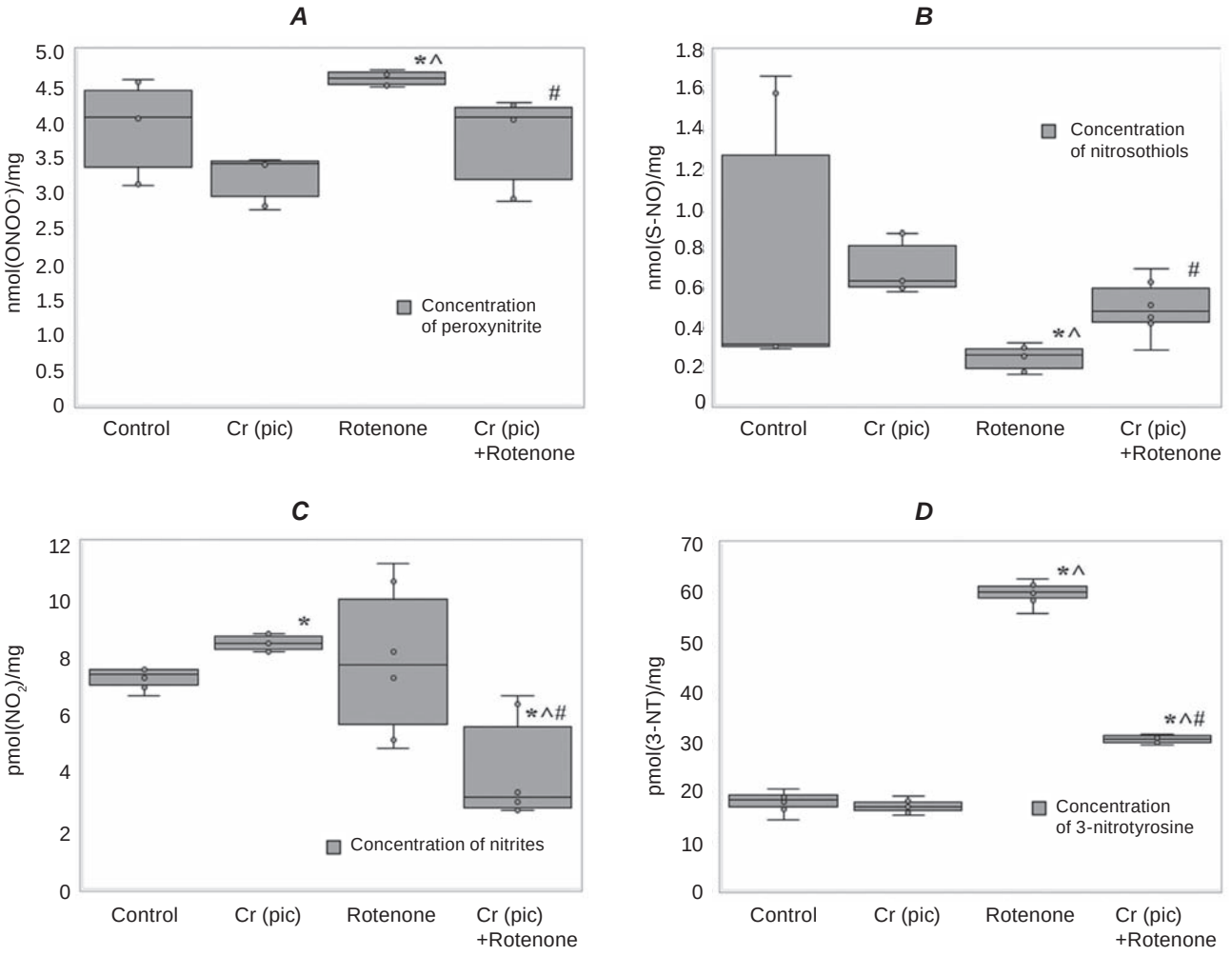

*Fig. 4. Metabolites of nitric oxide in the brain of rats under the conditions of correction of rotenone intoxication with chromium picolinate. **A** – the content of peroxynitrite, **B** – the content of nitrosothiols, **C** – the content of nitrites, **D** – the content of 3-nitrotyrosine. Notes: *P < 0.05 compared to the control group; ^P < 0.05 compared to the group administered chromium picolinate; #compared to the rotenone intoxication group. Symbols: Cr (pic) – chromium picolinate*

1.71 times compared to the control, 1.79 times compared to the group of animals administered chromium picolinate and decreased 1.95 times compared to the rotenone intoxication group ($P < 0.05$).

Oral administration of chromium picolinate to the body of animals does not lead to the development of oxidative-nitrosative damage to brain tissues. An increase in the activity of nitrate and nitrite reductase can be a consequence of the direct effect of the chromium cation on the activity of these enzymes [29]. It should also be noted the possible dependence of this effect on the content of chromium cations in the cell/organism, since at high contents chromium cations contribute to the oxidation of nitrites to nitrates [30]. Increased production of superoxide anion radical in brain tissues under the influence of oral administration of chromium picolinate to the body may result from chromium-induced generation of reactive oxygen species from the mitochondrial electron transport chain [31]. The increased activity of arginases may be related to the ability of chromium picolinate to activate the p38/MAPK cascade [32]. It is worth noting that there is a reciprocal relationship between the activation of the p38/MAPK cascade and the activity of arginases. According to the literature, a decrease in the activity of arginases leads to increased activation of the p38/MAPK cascade through p32 [33]. A decrease in the content of H_2S in brain tissues under the conditions of administration of chromium picolinate may be related to the ability of the chromium cation to interact with H_2S, which has been shown in studies on plants [34, 35].

A decrease in the activity of such antioxidant enzymes as catalase and superoxide dismutase in the brain of rats under the conditions of rotenone administration may be associated with a decrease in the activation of the transcription factor Nrf2, which controls the expression of antioxidant genes and is in an antagonistic relationship with the transcription factor NF-κB [36, 37]. In the current literature, there is no direct evidence of the ability of rotenone to inhibit the activation of the transcription factor Nrf2; however, most pharmacological means of influencing Nrf2 have a positive effect on the activity of antioxidant enzymes under conditions of rotenone intoxication [38, 39]. On the other hand, depletion of the antioxidant system can be observed in the brain tissues, which is due to the excessive production of reactive oxygen and nitrogen forms, which is observed with the introduction of rotenone in our study. Peroxynitrite can inactivate superoxide dismutase by nitrosylation. Therefore, a decrease in superoxide dismutase activity may be a direct consequence of the excess formation of peroxynitrite in brain tissues due to increased iNOS activity [40].

Rotenone can directly increase the production of superoxide anion radical (SAR) in brain tissues by inhibiting mitochondrial complex I [40, 41]. Also, SAR production can increase as a result of rotenone-induced activation of the transcription factor NF-κB, which leads to increased production of SAR by NADPH oxidases of macrophages [42, 43]. The development of hypoxia due to peroxynitrite-induced vasoconstriction can be a separate mechanism for increasing the production of SAR under the conditions of rotenone intoxication [44]. The increase in iNOS activity under the conditions of rotenone intoxication may be a consequence of the activation of the transcription factor NF-κB [41, 42].

Therefore, the increased production of reactive forms of oxygen and nitrogen under the conditions of rotenone intake leads to the development of oxidative-nitrosative damage to rat brain tissues, as evidenced by the increase in the content of MDA and 3-NT. Under these conditions, the predominant route of utilization of excess nitric oxide produced by iNOS is the formation of peroxynitrite, while the formation of nitrites and nitrosothiols decreases. A decrease in the activity of arginases in the brain of rats under the conditions of rotenone administration may be related to the competition between iNOS and arginases for the substrate of the reaction.

Our study also revealed a decrease in the activity of the nitrate-nitrite reductase pathway of nitric oxide formation in brain tissues under the influence of rotenone. A decrease in the content of sulfides in rat brain homogenate under the influence of rotenone may be related to the ability of rotenone to inhibit the activity of the enzyme cystathionine β-synthase [45, 46].

A decrease in SAR production in brain tissues with the combined intake of chromium picolinate and rotenone to the body may be associated with competition between chromium ions and rotenone for the ligand site in the mitochondrial complex I [47]. A decrease in the production of reactive oxygen species logically contributes to the improvement of the conditions for the functioning of antioxidant enzymes and reduces the intensity of lipid peroxidation.

Despite the ability of chromium picolinate to increase iNOS activity by activating the p38-MAPK/

NF-κB/iNOS pathway, an increase in the content of chromium ions in the cell can alter the activity of the NF-κB/iNOS pathway, so the decrease in iNOS activity in the combination group of rotenone and chromium picolinate may be associated with the accumulation of chromium ions [48, 49]. A decrease in iNOS activity naturally leads to a decrease in the content of 3-NT and peroxynitrite and contributes to an increase in the formation of nitrosothiols. As already mentioned above, chromium picolinate can increase nitrite reductase activity, which explains the decrease in nitrite content in the group of animals with the combined use of rotenone and chromium picolinate [29, 30]. The increase in the activity of arginases in rat brain tissues under the conditions of the combined intake of rotenone and chromium picolinate is associated with a decrease in the activity of iNOS in this group compared to the group of the isolated intake of rotenone, which releases the substrate for arginases.

In general, a decrease in sulfide content in the rotenone exposure group should be considered a negative event. Hydrogen sulfide is essential for direct brain antioxidant protection due to scavenging of free radicals and indirect antioxidant protection due to sulfide-induced activation of transcriptional factor Nrf-2 [50]. H_2S is also necessary for protein persulfidation of brain tissues. Therefore, an increase in H_2S content in the group with a combined intake of rotenone and chromium picolinate is a beneficial event for brain functioning. However, a decrease in sulfite content in the rotenone group may be viewed as a beneficial event given the toxicity of sulfites towards brain tissues [51]. Therefore, an increase in sulfite content in the group with combined intake of rotenone and chromium picolinate may indicate chromium picolinate's adverse effect and toxicity.

Conclusions. The introduction of rotenone into the body of rats is accompanied by the development of oxidative-nitrosative stress in brain tissue. Rotenone increases the production of active forms of nitrogen mainly due to the increase in the activity of the inducible isoform of NO-synthase. Under the influence of rotenone in the brain tissues of rats, there is a decrease in the content of H_2S and SO_3^{2-}.

Oral administration of chromium picolinate against the background of rotenone administration prevents the development of oxidative-nitrosative stress in brain tissue by reducing the production of reactive oxygen and nitrogen forms, promotes the restoration of arginase activity and increases the content of H_2S and SO_3^{2-}, which provides grounds for further study of its neuroprotective effect to prevent neurodegenerative disorders.

The limitation of our study is that we did not study the separate effects of chromium ions and picolinic acid on studied parameters during rotenone intoxication.

Conflict of interest. The authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

Funding. The authors received no funding for this article.

ПІКОЛІНАТ ХРОМУ ЗАПОБІГАЄ РОЗВИТКУ ОКСИДАТИВНО-НІТРОЗАТИВНОГО СТРЕСУ ТА ВІДНОВЛЮЄ ЕНДОГЕННУ ПРОДУКЦІЮ H_2S У МОЗКУ ЩУРА ЗА УМОВ РОТЕНОН-ІНДУКОВАНОЇ ТОКСИЧНОСТІ

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Енергодефіцит, мітохондріальна дисфункція та оксидативний стрес, спричинені ротеноном, можуть відігравати вирішальну роль у патогенезі нейродегенеративних розладів. Піколінат хрому продемонстрував нейропротекторну дію та ефективність у лікуванні хвороби Альцгеймера. Вплив піколінату хрому на мозок за умов впливу ротенону не досліджувався, і такі дані можуть пролити світло на патогенез нейродегенеративних захворювань. Мета дослідження – визначити вплив піколінату хрому на показники оксидативно-нітрозативного стресу та вміст сульфід-аніону та сульфітів у гомогенаті головного мозку за умов введення ротенону щурам. Експерименти проводили на 24 білих статевозрілих щурах самцях Вістар. Тварини були розподілені на 4 групи: контрольна; група піколіната хрому; група ротенону; група

комбінованого впливу піколіната хрому і ротенону. Піколінат хрому вводили перорально в дозі 80 мкг/кг на добу протягом 21 дня. Ротенон вводили підшкірно в дозі 1,5 мг/кг через день. Введення ротенону в організм щурів супроводжувалося розвитком оксидантно-нітрозативного стресу переважно за рахунок підвищення активності індуцибельної ізоформи NO-синтази та зниження вмісту H_2S і SO_3^{2-} в мозковій тканині. Зроблено висновок, що пероральний прийом піколінату хрому на тлі прийому ротенону запобігає розвитку оксидативно-нітрозативного стресу в тканинах головного мозку за рахунок зниження продукції активних форм кисню та азоту, сприяє відновленню активності аргіназ та підвищує вміст H_2S і SO_3^{2-}.

Ключові слова: піколінат хрому, ротенон, оксидативно-нітрозативний стрес, NO-синтаза, H_2S, мозок щура.

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