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ВЛИЯНИЕ ГАММА-ОБЛУЧЕНИЯ НА ЖИРНОКИСЛОТНЫЙ СОСТАВ ОБЩИХ ЛИПИДОВ КОЖИ КРОЛИКОВ

Установлено, что гамма-излучение приводит к достоверному снижения у шкуре кролей содержания таких жирних кислот, как меристиновая, пантадекановая, пальмитиновая, пальмитоолеиновая, линолевая, арахидоновая, а под конец исследваний, тоесть на 76-тые сутки, концентрация жирных кислот повышалась, а меристиновой и пальмитолеиновой приблизилась к физиологической норме.

Ключевые слова: гамма-радиация, кроли, жирные кислоты, шкура, пиридоксин

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INFLUENCE OF GAMMA IRRADIATION ON THE FATTY ACID COMPOSITION OF TOTAL LIPIDS RABBIT SKIN

It is found that gamma radiation leads to a significant decrease in the skin of rabbits content of these fatty acids, as meristinic, pantadekanovaya, palmitic, palmitoleic, linoleic, arachidonic, and at the end issledvany, ie 76 Tide day, the concentration of fatty acids increased, and meristinic palmitoleic and close to the physiological norm.

Key words: gamma-radiation, rabbits, lipids acids, skin, piridoxyn.

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ВПЛИВ НОВОГО ТІОСУЛЬФОНАТНОГО ПОХІДНОГО ХИНОНУ НА АДФ-ІНДУКОВАНУ АГРЕГАЦІЮ ТРОМБОЦИТІВ

Авторами виявлено, що S-((1,4-диметокси-9,10-діоксо-9,10-дигідроантрацен-2-іл)метил)-4-амінобензенсульфонотіоат володіє антитромбоцитарною активністю. Для з'ясування механізму його антиагрегаційної дії, провели серію додаткових експериментів. Було встановлено, що сполука інгібує АДФ-індуковану агрегацію тромбоцитів. Інгібуючий ефект залежить від концентрації препарату та часу преінкубації. Похідне також виявляє дисагрегаційну дію щодо агрегатів тромбоцитів, попередньо сформованих внаслідок АДФ-стимуляції.

Ключові слова: збагачена тромбоцитами плазма, тіосульфонатні похідні хінону, АДФ-індукована агрегація тромбоцитів, антитромбоцитарні засоби.

Introduction. Platelet activation and aggregation play a crucial role in the maintenance of normal hemostasis [1, 2], but malfunction of these processes can lead to a loss of hemostatic equilibrium within the blood vessel resulting in the formation of occlusive platelet-rich thrombi, responsible for the manifestations of atherothrombotic disease [3-5]. Pharmacologic modification of platelet function reduces the risk for the development of thrombotic diseases and their complications [6, 7]. Ideally, a clinically useful, platelet-modifying drugs should be nontoxic, orally effective, has sustained action and good antithrombotic potency without excessive risk of abnormal bleeding. None of the clinicaly available agents satisfy sufficient all these requirements. Moreover, despite the proven benefits of current antiplatelet agents, morbidity and mortality rates for thrombotic disease are remaining at high level. Therefore, there is much room for further improvement of antiplatelet treatment and development of novel antiplatelet agents with increased efficacy and safety profile.

Several studies have suggested that some quinone derivatives can significantly modify platelet functions [8, 9]. On the other hand, it was found that sulfur-rich compounds that contain $R\text{-}SO_2\text{-}CH_2\text{-}S\text{-}S\text{-}R$ fragment are also known as the agents with antithrombotic activity [10]. Taking into account these observations, we have synthesized derivatives, containing quinoid system of bonds and thiosulfonate fragment in one molecule. At the result of screening the synthesized derivatives for their antiplatelet activity [results in print], a novel small molecule – S-((1,4-dimethoxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl) 4-aminobenzenesulfonothioate (Fig. 1) – with high antiplatelet activity have been discovered. The studied compound at concentration of 100 μM had full inhibitory effect on ADP-induced platelet aggregation.

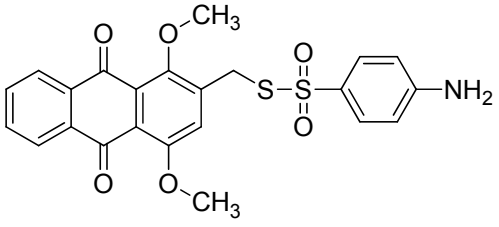

Fig. 1. Chemical structure of S-((1,4-dimethoxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl)4-aminobenzenesulfonothioate

The aim of the present study was to obtain more information about the effects of S-((1,4-dimethoxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl)4-aminobenzenesulfonothioate on platelet aggregation function and mechanism of its action.

Materials and methods. Studied compound, S-((1,4-dimethoxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl) 4-aminobenzenesulfonothioate, was synthesized at the Department of Technology of Biologically Active Substances, Pharmacy and Biotechnology, of Lviv Polytechnic National University. The compound was synthesized according the scheme depicted in Figure 2.

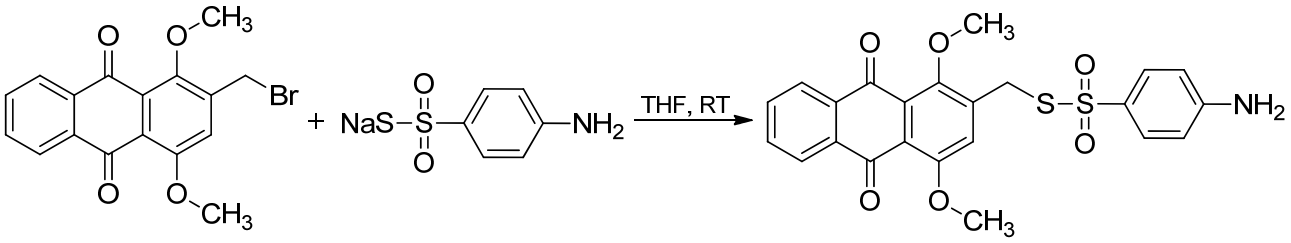

Fig.2. Synthesis of S-((1,4-dimethoxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl)4-aminobenzenesulfonothioate

Melting points were determined on a Büchi capillary melting point apparatus and are uncorrected. Element analyses were performed by the centre of Microanalyse of the Aix-Marseille University. Both ^{1}H and ^{13}C NMR spectra were determined on a Bruker AC 200 spectrometer. The ^{1}H the ^{13}C chemical shifts are reported from $CDCl_3$ peaks: ^{1}H (7.26 ppm) and ^{13}C (77.16 ppm) and from DMSO peaks: ^{1}H (2.50 ppm) and ^{13}C (39.52 ppm).

Silica gel 60 (Merck, particle size 0.063–0.200 mm, 70–230 mesh ASTM) was used for column chromatography. TLC was performed on 5 cm × 10 cm aluminum plates coated with silica gel 60 F254 (Merck) in an appropriate solvent.

Procedure for synthesis of S-((1,4-dimethoxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl)4-amino-benzenesulfonothioate:

Into two-necked flask equipped with a nitrogen inlet was added solution of 2-(bromomethyl)-1,4-dimethoxyanthracene-9,10-dione (0,77 g, 2,13 mmol) in THF (10 ml) and dissolved in portion of THF (10 ml) sodium salt of 4-amino-benzenethiosulfonic acid (0,45 g, 2,13 mmol). The solution was stirred and maintained at room temperature for 5 hours. After this time, TLC analysis showed that compound 2 was totally consumed. The reaction mixture was treated with ice water and extracted 3 times with dichloromethane. The organic phase was washed with water, and then dried over anhydrous sodium sulfate. After evaporation, product was purified by silica gel chromatography and recrystallized from ethanol, gave corresponding S-((1,4-dimethoxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl)4-aminobenzenesulfonothioate.

S-((1,4-dimethoxy-9,10-dioxo-9,10-dihydroanthracen-2-yl) methyl)4-amino-benzenesulfonothioate:

Yellow precipitate, Mp. 215-217 °C, yield 30 %.

^{1}H NMR (200 MHz, DMSO-d6) δ, ppm: 3.83 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 4.35 (s, 2H, CH_2), 6.09 (s, 2H, NH_2), 6.69 (d, *J* = 8.6 Hz, 2H, Ar-H), 7.28 (s, 1H, Ar-H), 7.67 (d, 2H, *J* = 8.6 Hz, Ar-H), 7.71-7.74 (m, 2H, Ar-H), 8.12-8.17 (m, 2H, Ar-H).

^{13}C-NMR (50 MHz, DMSO-d6) δ, ppm: 34.5 (CH_2), 56.8 (OCH_3), 62.7 (OCH_3), 114.0 (2CH), 120.4 (CH), 125.4 (C), 126.4 (CH), 126.6 (CH), 127.1 (C), 129.6 (CH), 133.0 (C), 133.4 (CH), 133.6 (C), 133.8 (2CH), 134.2 (C), 138.3 (C), 151.1 (C), 152.3 (C), 156.2 (C), 182.7 (CO), 183.0 (CO).

Calculated for ($C_{23}H_{19}NO_6S_2$), %: C 58.84; H 4.08; N 2.98; O 20.45; S 13.66.

Found, %: C 58.02; H 4.41; N 2.76; S 12.85.

Assay of antiplatelet activity:

Preparation of platelet rich plasma (PRP) was done according to the method described previously [11]. All procedures were conducted at room temperature. Blood was collected from the auricular artery of healthy rabbit into 3.8 % citrate in a ratio of 9:1 and than centrifuged at 150 g for 15 min in order to obtain PRP. The PRP was carefully removed and placed into a plastic tube. Platelet-poor plasma (PPP) was prepared by further centrifugation of the remained plasma at 1500 g for 40 minutes. Throughout all experiments, the platelet number was adjusted to $250x10^{9}$/L by diluting PRP with PPP. Platelet aggregation in PRP was recorded under constant stirring conditions (500 rpm) at 37°C for 10 min by aggregometer (AT-02, Belarus). The baseline value was set using PRP while PPP served as full transmittance control. PRP suspension was incubated with the studied compounds (final concentration: 5, 10, 25, 50 and 100 μM) or with dimethylsulphoxide (DMSO) alone for 2 minutes. To minimize the effect of DMSO, the solvent, on aggregation, the final concentration of DMSO was fixed at 1% (v/v). The aggregation was induced by adding ADP (final concentration: 5 μM) and monitored the change of light transmission for 8 minutes, measuring the maximal increase after the addition of the inducer. The concentration at which the test compound showed 50% inhibition was taken as the IC_{50}. To study the time-dependent inhibitory effect of the test quinoid thiosulfonate derivative on ADP-induced platelet aggregation, samples of PRP were preincubated with 50 μM of studied compound for 0, 2, 3, 5, 20, 40, 60 minutes at 37°C with continuous stirring. The aggregation was induced by adding 5 μM ADP and monitored for the maximal changes in light transmission. To investigate the effect of the test quinoid thiosulfonate derivative on platelet disaggregation, samples of PRP were stimulated with ADP (5 μM) at 37°C with continuous stirring for the formation of aggregates in PRP. Studied compound (50, 100 μM) or 1% DMSO alone were added 90 seconds after the addition of inducer and changes in light transmission were recorded by aggregometer. Results were expressed as mean±SEM. The difference between groups was analyzed by standard Student's *t*-test. *P* values less than 0.05 were considered statistically significant.

Results and discussion. Platelet function can be regulated by various agonists. A major signaling molecule causing platelet aggregation is adenosine-5′-diphospate (ADP) which activates platelets and is known to play an important role in hemostasis and thrombosis. Moreover, ADP receptor antagonists are in wide clinical use [12, 13]. Therefore, we investigated the effect of the test derivative on ADP-induced platelet aggregation. Concentration of ADP which inducing maximal aggregation was determined in preliminary experiments. In rabbit platelet-rich plasma (PRP), the maximal changes in light transmission observed at the ADP (5 μmol/L) was 46±4%. In the platelet aggregation assay, samples of citrated PRP were preincubated with vehicle 1% DMSO (control) or with increasing concentrations of studied compound (5, 10, 25, 50, 100 μM) and than stimulated with

ADP to induce aggregation. Obtained results suggested that 1% DMSO had no effect on platelet aggregation induced by ADP. Test agent inhibited ADP-induced aggregation and the degree of inhibition was proportional to its concentration (Figure 3). As shown in Figure 3 the inhibition increased linearly from 5 to 100 μM with the half maximal inhibitory concentration (IC_{50}) – 50 μM.

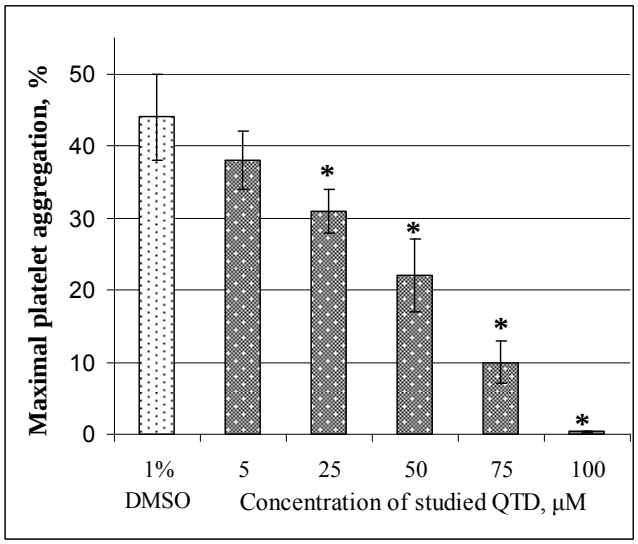

Fig. 3. Maximal ratio of ADP-induced platelet aggregation after 2 min incubation of PRP with different concentration of studied quinoid thiosulfonate derivative (QTD) or with 1% DMSO (M±m, n=6)

** – P<0.05 vs 1% DMSO*

The inhibitory effect of studied compound was inversely associated with preincubation time (Figure 4). For this experiment, samples of PRP were preincubated with 50 μM of test quinoid thiosulfonate derivative for 0, 2, 3, 5, 20, 40, 60 min and than stimulated with ADP (5 μM) to induce aggregation. As shown in Figure 4 the inhibition levels of ADP-induced aggregation observed after preincubation of PRP with 50 μM of compound for 0, 2, 3 or 5 minutes did not differ from each other. On the other hand, the inhibitory effect of S-((1,4-dimethoxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl) 4-aminobenzenesulfonothioate was significantly reduced after more than twenty minutes of incubation. Moreover, after preincubation of PRP with 50 μM of the derivative for 60 minutes, the level of aggregation was identical to that in untreated PRP (Figure 4).

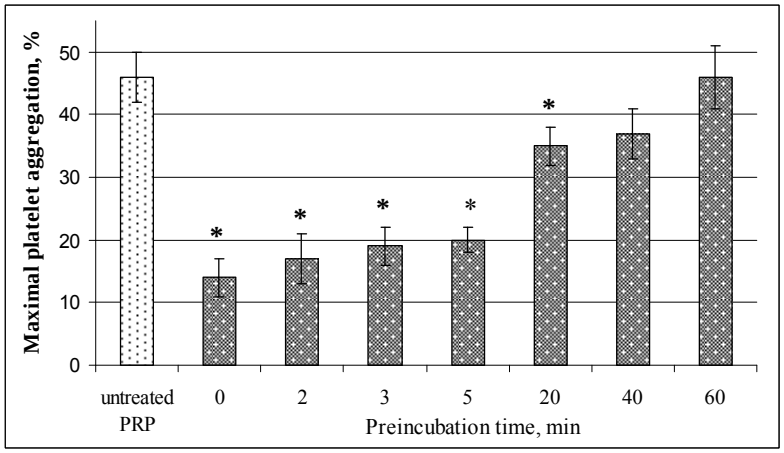

Fig. 4. Maximal ratio of ADP-induced platelet aggregation after preincubation of PRP with 50 μM of studied quinoid thiosulfonate derivative for 0, 2, 3, 5, 20, 40, 60 min (M±m, n=5)

** – P<0.05 vs untreated PRP*

Our results suggest that test compound at the concentration 100 μM could also effectively disaggregate the preformed platelet aggregate caused by ADP as the inducer. As shown in Figure 5 where PRP was incubated with ADP for 90 seconds, as soon as studied compound was added into the mixture, disaggregation occurred rapidly and profoundly. In contrast, the addition of lower concentration of tested derivative of thiosulfonate (50 μM) as well as vehicle 1% DMSO did not affect the aggregate (Figure 5).

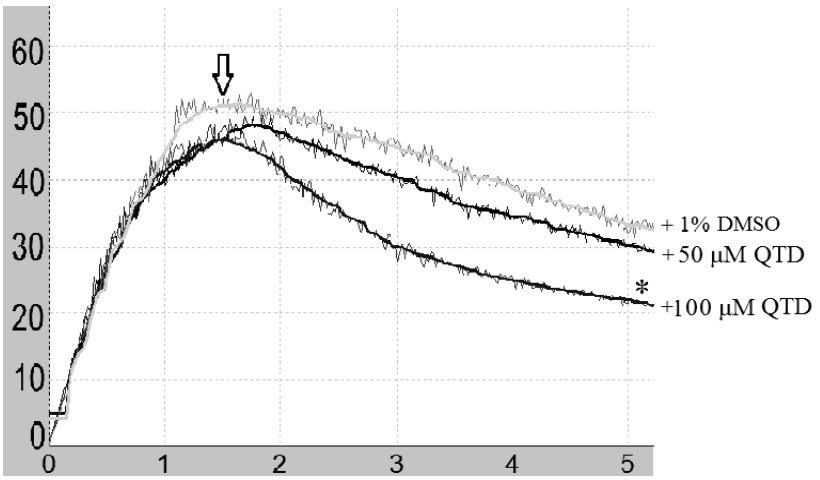

Fig. 5. Effect of 50 or 100 μM of studied quinoid thiosulfonate derivative (QTD) on platelet disaggregation

The moment of adding of effector into the ADP-stimulated PRP is indicated in Figure by the arrow

** – P<0.05 vs 1% DMSO-treated PRP*

Platelet aggregation is a result of complex signal transduction cascade reactions brought about by stimulants. One of the components in the cascade is ADP that is an important mediator of platelet aggregation and activation. ADP elicits its effects on the platelet through the membrane bound P2Y1 and P2Y12 receptors [14]. The P2Y1 receptor is coupled to Gq which regulates phospholipase C and intracellular Ca2+ mobilization and leads to aggregation and shape change. P2Y12 is coupled to Gi and leads to subsequent inhibition of adenylate cyclase and regulation of phosphoinositide 3-kinase. The importance of ADP in the process of thrombus formation has been demonstrated both by antiplatelet drugs that target the ADP receptors [14] and by patients with dysfunctional P2Y1/P2Y12 receptors [15]. ADP receptors may be important therapeutic targets. Moreover ADP receptor antagonists are in wide clinical use [11-13, 16-18]. However, several limitations of known synthetic ADP receptors antagonist have recently been discussed including inter-patient variability in antiplatelet effects and a relatively slow onset of action [19-21]. So, additional studies are needed and new selective platelet inhibitors with increased anti-thrombotic efficiency and safety profile must be developed. The results reported here indicate that S-((1,4-dimethoxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl) 4-aminobenzenesulfonothioate can inhibit ADP-induced effects on platelet aggregation. But detailed mechanism of its action deserves additional investigations. Hopefully, our further research may give rise to numerous new selective platelet inhibitors with increased anti-thrombotic efficiency and safety profile.

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ВЛИЯНИЕ НОВОГО ТИОСУЛЬФОНАТНОГО ПРОИЗВОДНОГО ХИНОНА НА АДФ-ИНДУЦИРОВАННУЮ АГРЕГАЦИЮ ТРОМБОЦИТОВ

Авторами обнаружено, что S-((1,4-диметокси-9,10-диоксо-9,10-дигидроантрацен-2-ил)метил)-4-аминобензенсульфонотиоат владеет антитромбоцитарной активностью. Для выяснения механизма его антиагрегационного действия, провели серию дополнительных экспериментов. Было установлено, что соединение ингибирует АДФ-индуцированную агрегацию тромбоцитов. Ингибирующий эффект зависит от концентрации препарата и времени преинкубации. Производное также оказывает дисагрегационное действие на агрегаты тромбоцитов предварительно сформированные в результате АДФ-стимуляции.

Ключевые слова: обогащенная тромбоцитами плазма, тиосульфонатные производные хинона, АДФ-индуцированная агрегация тромбоцитов, антитромбоцитарные средства.

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EFFECTS OF NOVEL QUINOID THIOSULFONATE DERIVATIVE ON ADP-INDUCED PLATELET AGGREGATION

Recently, in a large scale screening test, we have found that S-((1,4-dimethoxy-9,10-dioxo-9,10-dihydroanthracen-2-yl)methyl)4-aminobenzenesulfonothioate, a chemically synthesized thiosulfonate derivative of quinone, possessed an antiplatelet activity. To elucidate the mechanism of its antiplatelet action, a series of experiments were performed. The compound was found to inhibit the ADP-induced platelet aggregation. The inhibitory effect was dose-dependent on concentration and preincubation time. The derivative also disaggregated the preformed platelet aggregates induced by ADP.

Key words: platelet rich plasma, quinoid thiosulfonate derivative, ADP-induced platelet aggregation, antiplatelet agents.

УДК 591.147; 612.4

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ВПЛИВ МЕЛАТОНІНУ НА ГІПОТАЛАМО-АДРЕНАЛОВУ СИСТЕМУ ПТАХІВ: ЕФЕКТ БЛОКАДИ ДОФАМІНОВИХ D1-РЕЦЕПТОРІВ

Показано, що ввечері та вдень (але не вночі та вранці) дофамін через D1-рецептори може опосередковувати вплив мелатоніну на гіпоталамо-адреналовий комплекс птахів. Також дофамін через D1-рецептори може бути залучений до шляхів впливу мелатоніну на циркадну систему (супрахіазматичне ядро та епіфіз) ввечері, але ці рецептори не залучені вночі, вранці та вдень.

Ключові слова: наднирники, гіпоталамус, епіфіз, мелатонін, дофамін, D1-рецептори.

Вступ. Циркадні ритми відіграють значну роль в життєдіяльності тваринного організму. Важливим компонентом системи, що задає добову ритміку в організмі хребетних, є епіфіз. Епіфіз секретує мелатонін з добовою періодичністю, задаючи цим самим добовий ритм роботи інших органів та систем органів [3]. З поміж систем органів найбільш важливим є вплив мелатоніну на нейроендокринну систему, оскільки від її регуляторних впливів залежить злагоджена робота всіх інших систем в організмі. Однією зі складових нейроендокринної системи є гіпоталамо-гіпофізарно-адреналова система. На сьогодні завдяки численним дослідженням багато відомо про особливості впливу мелатоніну на гіпоталамо-гіпофізарно-адреналову систему [3, 5, 9, 10]. Проте спірні моменти залишаються, і не всі шляхи впливу цього епіфізарного гормона є до кінця з'ясованими. Зокрема, дослідження останніх років показали, що частина ефектів мелатоніну на гіпоталамо-гіпофізарно-адреналову систему може бути пов'язана з його впливом на синтез і виділення дофаміну, а останній вже діє безпосередньо через свої рецептори [4, 6, 7, 8, 11]. Для з'ясування можливості такої дії мелатоніну та дослідження ролі в цьому процесі дофамінових D1-рецепторів і проведено дане дослідження. Враховуючи наявність добової динаміки синтезу мелатоніну дослідження були проведені в 4 різних часових проміжках доби: вранці, вдень, ввечері та вночі.

Матеріали та методи досліджень. Дослідження було проведено на самцях японських перепелів (Coturnix coturnix japonica) 5-тижневого віку. Птахів утримували в умовах одного віварію на стандартному раціоні (їжа – комбікорм пташиний виробництва Київського комбікормового заводу, вода – ad libitum, температура – +22-23 ^{o}C). Світловий режим: 14 годин – світло (з 7 до 21 години), 10 годин – темрява (з 21 до 7 години).

Було сформовано 16 експериментальних груп по 5 птахів у кожній групі. Їм у відповідний час доби одноразово давали: 1.) фізіологічний розчин вранці (о 7:00) (контрольна група); 2.) мелатонін вранці (о 7:00) в дозі 10 мкг (тут і далі всі дози вказані з розрахунку на 100 г маси тіла); 3.) R(+)SCH 23390 гідрохлорид (блокатор дофамінових D1-рецепторів) вранці (о 6:00) в дозі 8 мкг; 4.) R(+)SCH 23390 гідрохлорид вранці (о 6:00) в дозі 8 мкг, а через годину (о 7:00) в дозі 10 мкг; 5.) фізіологічний розчин вдень (о 13:00) (контрольна група); 6.) мелатонін вдень (о 13:00) в дозі 10 мкг; 7.) R(+)SCH 23390 гідрохлорид вдень (о 12:00) в дозі 8 мкг; 8.) R(+)SCH 23390 гідрохлорид вдень (о 12:00) в дозі 8 мкг, а через годину (о 13:00) мелатонін в дозі 10 мкг; 9.) фізіологічний розчин ввечері (о 19:00) (контрольна група); 10.) мелатонін ввечері (о 19:00) в дозі 10 мкг; 11.) R(+)SCH 23390 гідрохлорид ввечері (о 18:00) в дозі 8 мкг; 12.) R(+)SCH 23390 гідрохлорид ввечері (о 18:00) в дозі 8 мкг, а через годину (о 19:00) мелатонін у дозі 10 мкг; 13.) фізіологічний розчин вночі (о 1:00) (контрольна група); 14.) мелатонін вночі (о 1:00) в дозі 10 мкг; 15.) R(+)SCH 23390 гідрохлорид вночі (в 0:00) в дозі 8 мкг; 16.) R(+)SCH 23390 гідрохлорид вночі (в 0:00) в дозі 8 мкг, а через годину (о 1:00) мелатонін у дозі 10 мкг. Фізіологічний розчин вводили перитонеально в кількості 0,2 мл на птаха. R(+)SCH 23390 гідрохлорид розчиняли у фізіологічному рочині і вводили за допомогою стереотаксичного приладу в порожнину третього шлуночка мозку. Мелатонін замішували у борошняні