THE STUDY OF THE PROTECTIVE EFFECT OF MITOCHONDRIAL UNCOUPLERS DURING ACUTE TOXICITY OF THE FUNGICIDE DIFENOCONAZOLE IN DIFFERENT ORGANS OF MICE

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Pesticides represent a serious problem for agricultural workers due to their neurotoxic effects. The aim of this study was to evaluate the ability of pharmacological oxidative phosphorylation uncouplers to reduce the effect of the difenoconazole fungicide on mitochondrial DNA (mtDNA) of various organs in mice. Injections of difenoconazole caused cognitive deficits in mice, and the protonophore 2,4-dinitrophenol (2,4-DNP) and Azur I (AzI), a demethylated metabolite of methylene blue (MB), prevented the deterioration of cognitive abilities in mice induced by difenoconazole. Difenoconazole increased the rate of reactive oxygen species (ROS) production, likely through inhibition of complex I of the mitochondrial respiratory chain. After intraperitoneal administration of difenoconazole lungs, testes, and midbrain were most sensitive to the accumulation of mtDNA damage. In contrast, the cerebral cortex and hippocampus were not tolerant to the effects of difenoconazole. The protonophore 2,4-DNP reduced the rate of ROS formation and significantly reduced the amount of mtDNA damage caused by difenoconazole in the midbrain, and partially, in the lungs and testes. MB, an alternative electron carrier capable of bypassing inhibited complex I, had no effect on the effect of difenoconazole on mtDNA, while its metabolite AzI, a demethylated metabolite of MB, was able to protect the mtDNA of the midbrain and testes. Thus, mitochondria-targeted therapy is a promising approach to reduce pesticide toxicity for agricultural workers.

Key words: difenoconazole; methylene blue; 2,4-dinitrophenol; azur I; mitochondrial DNA; uncoupled respiration

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INTRODUCTION

The widespread use of pesticides is one of the factors contributing to the significant increase in agricultural productivity over the last 100 years, which has made possible such rapid population growth in the world. However, the presence of pesticides in the environment, their chemical stability and ability to accumulate in the body represent a serious threat to fish, birds, pollinating insects, and human health [1].

Many pesticides are mitochondria-targeted agents; they are capable of inhibiting the mitochondrial electron transport chain (ETC), disrupting oxidative phosphorylation, and stimulating apoptosis and inflammation [2]. All these processes cause mitochondrial dysfunction, which is often the cause of many chronic diseases. Epidemiological studies have documented that people directly working with pesticides have an increased risk of developing such diseases as cancer, Parkinson's disease, cardiac, neurological, and reproductive disorders [3].

It is known that plant mitochondria are much less susceptible to damage as compared to animal mitochondria. Perhaps the reason for the stability of plant mitochondria is determined by existing alternative pathways, responsible for uncoupled respiration [4]. Transgenic animal models expressing alternative NADH dehydrogenases [5] or alternative oxidases have been repeatedly shown to exhibit resistance to various mitochondria-targeted inhibitors. Therefore, in this study, we propose a way to reduce pesticide toxicity by uncoupling mitochondrial oxidative phosphorylation.

2,4-Dinitrophenol (2,4-DNP) is a protonophore that transports of protons back into the mitochondrial matrix without ATP synthesis. Previously, 2,4-DNP was used in medicine as a biological additive. After the discovery of some toxic properties, it was banned for use in a number of countries [7]. However, it probably could be used as an antidote for pesticide poisoning. Methylene blue (MB) provides alternative electron transport, bypassing damaged or inhibited components of the ETC. This agent may be a mimetic of uncoupled respiration, as in transgenic animal models expressing alternative oxidases [8]. Azur I (AzI) is a demethylated metabolite of MB, characterized by the ionization state of the oxidized form. Oxidized AzI can assume a neutral quinoneimine form, which easily diffuses through membranes [9].

The aim of this study was to investigate the effect of one of the most common fungicides, difenoconazole, on mitochondrial toxicity in various organs during acute poisoning of the body and in *in vitro* models, as well as to evaluate the ability of MB, AzI, and 2,4-DNP

to neutralize the toxic effect of difenoconazole. To assess the degree of mitochondrial toxicity of the pesticide, we studied the amount of mtDNA damage, which is a reliable marker of oxidative stress in mitochondria.

MATERIALS AND METHODS

Animals

Two-month-old C57Bl/6 mice, obtained from the Stolbovaya nursery (A branch of the Scientific Center for Biomedical Technologies, Russia) were used in experiments. The animals were kept under standard vivarium conditions at 25°C, relative air humidity of at least 40%, a 12-h light/dark cycle, and had free access to food and water.

The Experimental Plan

Forty five mice used in the experiment *in vivo* were randomly subdivided into five groups. Animals of the control group (n=11) received a standard diet and water for 28 days and, on day 22 were injected intraperitoneally (i.p.) with saline (Solopharm, Russia). Animals of the second group (n=8) received a standard diet and water for 28 days, and on day 22 of the experiment they were i.p. injected with 1 g/kg difenoconazole (TM Rayok; Avgust, Russia). Mice of the third group (n=9) received a standard diet and water with the addition of 2,4-DNP (Sigma-Aldrich, USA; a daily dose of 15 mg/kg). Animals of the fourth group (n=8) received a standard diet and water with the addition of MB (Sigma-Aldrich; a daily dose of 15 mg/kg). Mice of the fifth group (n=9) received a standard diet and water with the addition of AzI (Interchem, Russia; a daily dose of 15 mg/kg). On day 22 of the experiment, animals of the third, fourth, and fifth groups were i.p. injected with 1 g/kg difenoconazole (Table 1).

On days 24–28 of the experiment, the T-maze test was performed (Table 1). On day 29 of the experiment, the animals were sacrificed by rapid cervical dislocation followed by decapitation, and for the molecular part of the experiment, the cerebral cortex, hippocampus, midbrain, cerebellum, lungs, heart, liver, kidneys, and testes were excised.

Physiological Tests

Cognitive parameters have been evaluated using the standard T-maze test protocol described previously [10]. The test was carried out from day 24 to day 28 of the experiment. The first day was the habituation phase: animals were placed in a maze for 15 min, while pieces of food were randomly scattered throughout the maze at a short distance from each other so that the animals knew that there was food in the maze. The habituation stage continued during the next day: animals were placed in a maze for 15 min, but the food was in two opposite arms of the maze. Tests were carried out on days 3–5. The first trial for each animal was a training trial. During training, a reward was placed in each hand, but one remained closed. The animal had to enter the open hand and completely eat the reward. The second attempt was performed immediately, without a five-minute break. An experimenter opened the previously closed hand, and if the animal entered the hand where it had not previously been, it received a reward. On the next trial, the hand containing the reward was changed. If the animal did not fall into the correct hand, then on the next trial the reward remained in the same hand. A total of 10 attempts were made per day at five-minute intervals. The number of correct attempts corresponded to the number of points (minimum 0, maximum 10).

Measurement of Mitochondrial Respiration

Intact brain mitochondria isolated separately from the cortex and midbrain of mice according to the method described previously were used for *in vitro* experiments [11]. Mitochondria were isolated using digitonin fractionation. The homogenization buffer contained 200 mM mannitol, 75 mM sucrose, 20 mM HEPES (pH 7.4), 1 mM EGTA, and 2 mg/ml fatty acid-free bovine serum albumin (BSA). The wash buffer had the same composition, but without BSA. Mouse brain regions were homogenized using a Downs-type homogenizer. The homogenate was centrifuged for 5 min at 900 *g*. The supernatant was transferred into clean tubes and centrifuged for 10 min at 14,000 *g*. The supernatant was then removed and the mitochondrial pellet

Table 1. The time-scale of the experiment

Group / Day	Control (ordinary drinking water from day 1 to day 29)	Dif (n=8) (ordinary drinking water from day 1 to day 29)	2,4-DNP+Dif (n=9) (water supplemented with 2,4-DNP at a daily dose 15 mg/kg, from day 1 to day 29)	MB+Dif (n=8) (water supplemented with MB at a daily dose 15 mg/kg, from day 1 to day 29)	AzI+Dif (n=9) (water supplemented with AzI at a daily dose 15 mg/kg, from day 1 to day 29)
22	Saline injection	Dif injection (1 g/kg)	Dif injection (1 g/kg)	Dif injection (1 g/kg)	Dif injection (1 g/kg)
24–28	T-maze	T-maze	T-maze	T-maze	T-maze
29	Euthanasia	Euthanasia	Euthanasia	Euthanasia	Euthanasia

Dif – Difenoconazole, 2,4-DNP – 2,4-Dinitrophenol, MB – methylene blue, AzI – Azur I.

resuspended in the tubes with wash buffer and then incubated with 0.2% digitonin for 2 min on ice. The tubes were centrifuged for 15 min at 14,000 *g*. The supernatant was removed, and the sediment was washed twice in wash buffer by centrifugation for 10 min at 14,000 *g*. The final pellet was resuspended in 100 μl of wash buffer. The protein content in the resultant mitochondrial suspension was determined using the Pierce™ BCA Protein Assay Kits (Thermo Fisher Scientific, USA). The rate of mitochondrial respiration was measured using a Clark type electrode (Hansatech Instruments, UK). All measurements were performed in mitochondrial isolation buffer with 4 mM KH_2PO_4, respiratory substrates (5 mM pyruvate + 5 mM malate) and 50 μg of mitochondrial protein. The respiration rate was expressed as the oxygen consumption rate (nmol/min/mg protein). Difenoconazole was added at a concentration of 20 μM.

Measurement of Membrane Potential

The membrane potential was monitored by safranin O fluorescence (Sigma-Aldrich) (excitation and emission wavelengths of 495 nm and 586 nm, respectively) using a Hitachi F-7000 spectrofluorometer (Hitachi High Technologies, Japan). The incubation medium (1 ml) contained buffer A, 2 μM safranin O, 4 mM KH_2PO_4, respiratory substrates (5 mM pyruvate + 5 mM malate), and 50 μg of mitochondrial protein. The membrane potential was recorded in the absence and presence of 2 mM ADP. Difenoconazole was added at a concentration of 20 μM. Complete loss of membrane potential was achieved by adding 1 μM 2,4-DNP.

Measurement of H_2O_2 Release

The rate of H_2O_2 release was measured using a Hitachi F-7000 fluorescence spectrophotometer. The H_2O_2 concentration was determined by the fluorescence intensity of resorufin formed during the reaction initiated by adding 1 U Amplex UltraRed (Invitrogen, USA) and 4 U horseradish peroxidase (ThermoFisher Scientific). The excitation wavelength was 568 nm, the emission wavelength was 581 nm. All measurements were carried out in an acrylic cuvette in mitochondria isolation buffer. For each test compound, at least 3 independent measurements were performed.

Measurement of the Amount of mtDNA Damage

DNA extraction was performed using the PREP-GS DNA Extraction Kit (DNA-Technology, Russia). The amount of mtDNA damage was assessed using long fragment PCR; the protocol and primers were described previously [12].

Statistical Analysis

Statistical analysis was performed using Statistica 12. Results were expressed as mean ± standard error of the mean (M ± SEM). Data were analyzed using one-way analysis of variance (ANOVA). Tukey's *post hoc* test was used to determine the level of significance. Quantitative evaluation of mtDNA damage was performed using the DNADamageCalculator program (Voronezh, Russia).

RESULTS AND DISCUSSION

Administration of difenoconazole caused cognitive impairments in mice, which were registered in the T-maze test (Fig. 1). The T-maze test score of difenoconazole-treated mice was 17% lower than control animals treated with saline ($p<0.05$). The combined administration of difenoconazole with 2,4-DNP and AzI but not MB abolished the effect of this fungicide (Fig. 1).

Difenoconazole caused mtDNA damage in all the organs studied. According to the degree of mtDNA damage (from maximum to minimum), the organs are arranged in the following order: lungs (+104% damage (increase from 2.5±0.3 in the group of mice treated with saline to 5.0±0.2 in the group of difenoconazole-treated mice), $p<0.001$), testes (+57% (from 2.4±0.2 to 3.7±0.3), $p<0.001$), liver (+36% (from 2.2±0.3 to 3.1±0.3), $p=0.08$), heart (+30% (from 4.1±0.5 to 5.4±0.3), $p<0.05$), kidneys (+14% (from 4.9±0.5 to 5.6±0.2)), brain (+9% (from 3.7±0.2 to 4.1±0.2)) (Fig. 2A). Thus, the lungs are the most sensitive organ to the effects of this fungicide. It is well known that occupational exposure to pesticides is associated with an increased risk of developing respiratory symptoms, asthma, and chronic bronchitis [13]. In our acute experiment, difenoconazole was i.p.-injected, but even with this method of administration to the body, it caused severe damage to mtDNA in the lungs (Fig. 2A, 3B).

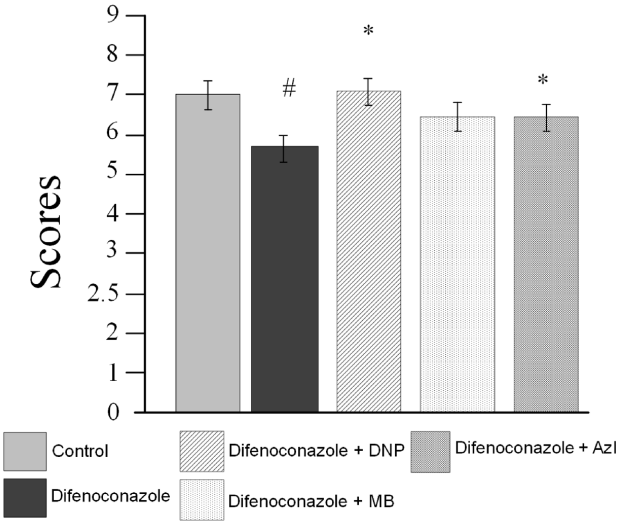

Figure 1. Results of the T-maze test. * $p<0.05$ – differences are statistically significant as compared to animals treated with difenoconazole. # $p<0.05$, ### $p<0.001$ – differences are statistically significant as compared to animals treated with saline.

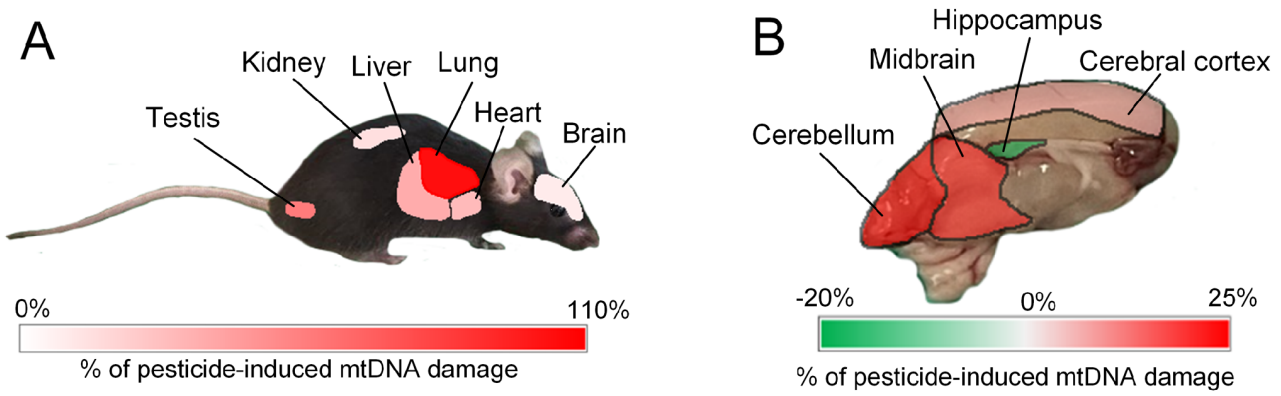

Figure 2. The extent of difenoconazole-induced mtDNA damage in internal organs (**A**) and brain regions (**B**). The color version of this figure is available in the electronic version of the article.

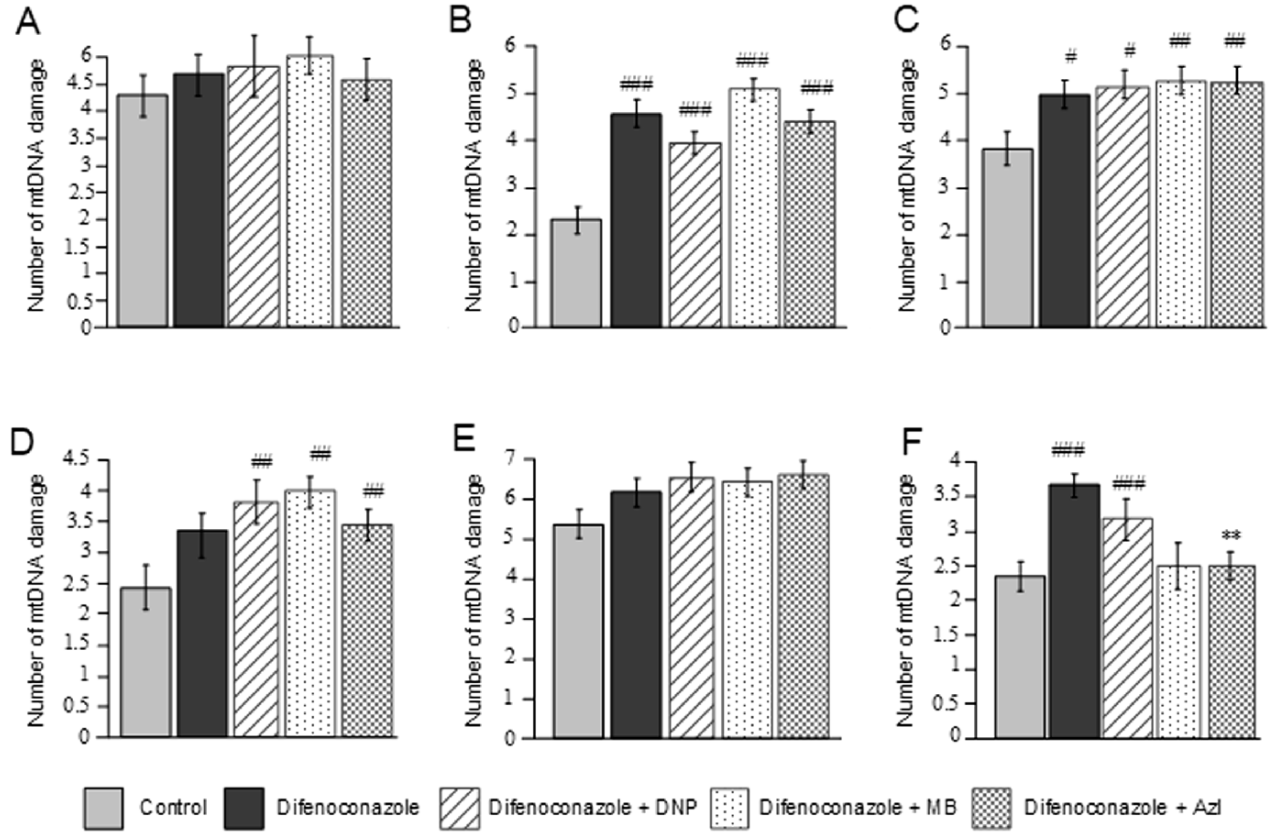

Figure 3. The amount of mtDNA damage in the studied organs: (**A**) brain, (**B**) lungs, (**C**) heart, (**D**) liver, (**E**) kidneys, (**F**) testes. All measurements were performed for at least six mtDNA fragments. ** $p<0.01$ – differences are statistically significant as compared to animals treated with difenoconazole; # $p<0.05$, ## $p<0.01$, ### $p<0.001$ – differences are statistically significant as compared to animals treated with saline.

The lungs are a highly specialized organ that absorbs oxygen, which, in the presence of toxicants, can become a source of ROS [14]. It is likely that the difenoconazole-induced doubling of mtDNA damage in the lungs is a consequence of increased oxidative stress.

Severe difenoconazole-induced damage was observed in the testes (Figs. 2A, 3F). These results are consistent with the data that chronic exposure of rats to difenoconazole reduced sperm quality, damages DNA, and decreased serum levels of testosterone, luteinizing hormone, and follicle-stimulating hormone [15]. Results of population-based studies suggest that long-term exposure to pesticides may be associated with certain reproductive problems observed over a decade later [16].

In the liver, we also found an increase in damage by 36%, but the differences did not reach the level of statistical significance (p=0.08) (Fig. 3D). Some studies have shown that exposure to difenoconazole caused hepatotoxicity and liver degeneration in fish larvae, as well as necrosis and neoplasms in the liver [17].

Difenoconazole injections had serious effects on cardiac mtDNA (Figs. 2A, 3C). Previous studies have shown that exposure to difenoconazole may cause cardiovascular diseases. The accumulation of ROS contributed to the development of oxidative stress in the heart. In general, difenoconazole-induced cardiovascular toxicity results in apoptosis and inhibition of cardiac muscle contraction [18].

In the kidneys, we did not find a statistically significant increase in the number of mtDNA damage after exposure to difenoconazole (Fig. 2A, 3E).

Overall, our data showed that brain mtDNA was less susceptible to the damaging effect of difenoconazole injection compared to other organs. There was a 9% increase in the number of lesions, but the difference was not significant (p=0.11). These data contradict previous studies that showed that difenoconazole disrupted the blood-brain barrier, caused ROS accumulation, inflammation and inhibited defense signaling pathways (particularly, the Nrf2/ARE pathway) in fish [19]. Difenoconazole caused serious mtDNA damage in the brain of bumblebees both through contact exposure and when it was added to nutrient syrup [20]. Nevertheless, our data show that brain mtDNA is less susceptible to oxidative damage compared to internal organs (Fig. 2A), although it is worth considering that the brain is a structurally heterogeneous organ and different parts of the brain are susceptible to pesticide damage to varying degrees. Indeed, we showed that difenoconazole injections caused severe damage to midbrain mtDNA (+22% (from 4.7±0.3 to 5.7±0.2), p<0.05). The difenoconazole-induced increase in mtDNA damage in the cerebellum (+25% (from 3.1±0.4 to 3.9±0.3), p=0.11) and cerebral cortex (+8% (from 2.8±0.3 to 3.1±0.2), p=0.38) was not statistically significant (Fig. 4).

Thus, it can be assumed that the midbrain is more susceptible to difenoconazole-induced damage. To test this hypothesis, we treated intact mitochondria obtained from the midbrain and cerebral cortex with various concentrations of difenoconazole. Difenoconazole caused mtDNA damage in the cerebral cortex only at a concentration of 200 μM, whereas in the midbrain it significantly damaged mtDNA even at a concentration 10 times lower (Fig. 5).

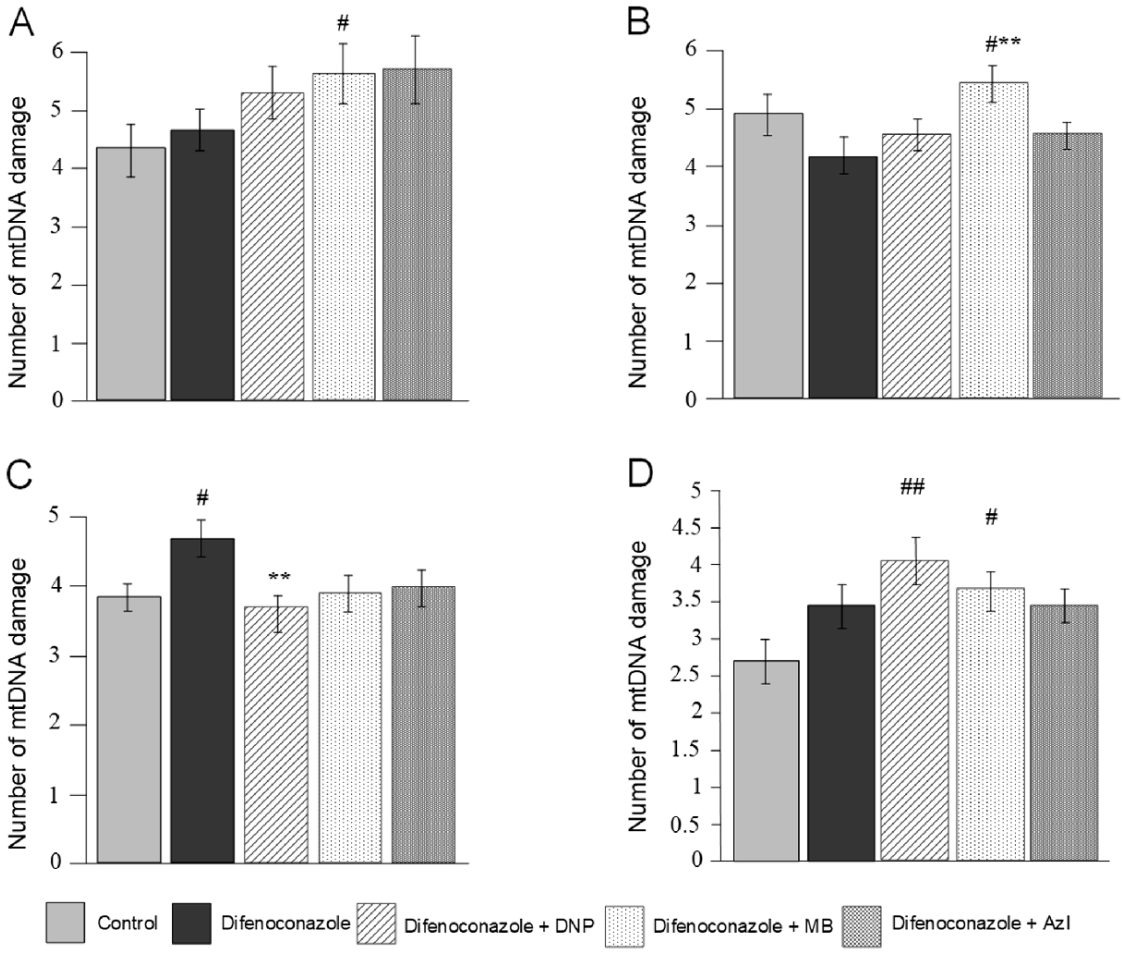

Figure 4. The effect of 2,4-DNP, MB, and AzI on the amount of difenoconazole-induced mtDNA damage in brain regions: (**A**) cerebral cortex, (**B**) hippocampus, (**C**) midbrain, (**D**) cerebellum. All measurements were performed for at least six mtDNA fragments. ** p<0.01 – differences are statistically significant as compared to animals treated with difenoconazole. # p<0.05, ## p<0.01 – differences are statistically significant as compared to animals treated with saline.

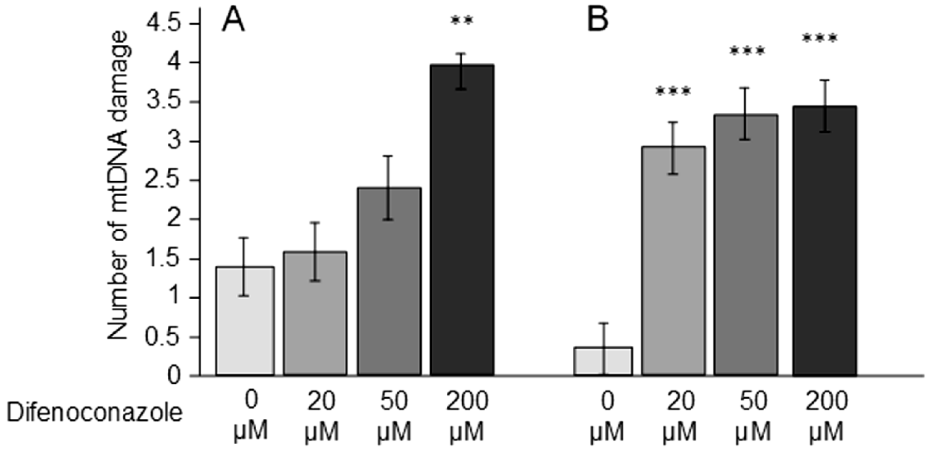

Figure 5. The *in vitro* effect of various concentrations of difenoconozole on the amount of mtDNA damage in the cerebral cortex (**A**) and midbrain (**B**). All measurements were performed for at least six mtDNA fragments. ** $p<0.01$, *** $p<0.001$ – the differences are statistically significant as compared to control mitochondria.

Previously, similar data were obtained in the study of the rotenone effect on mtDNA damage in the brain. Injections of rotenone resulted in an increase in the amount of mtDNA damage in the midbrain, but not in the cerebral cortex [21]. Good evidence exists that the midbrain accumulates more oxo8dG (a marker of oxidative DNA damage) than, for example, the cerebral cortex, and this is associated with glycosylase activity responsible for the removal of oxo8dG [22]. It has been shown that the rate of O_2^- generation in ventral mesencephalic cell cultures treated with low concentrations of rotenone is significantly higher than in cerebral cortex cell cultures [23]. It is suggested that this sensitivity of the midbrain is due to the characteristics of dopamine neurons. Dopaminergic neurons exhibit rhythmic electrical activity; they are exposed to increased oxidative stress, presumably associated with a high rate of dopamine synthesis [24]. Oxidative deamination of dopamine by monoamine oxidases produces H_2O_2. In addition, dopamine oxidation can occur through interaction with labile iron, which is necessary for the Fenton reaction and the formation of $^{\bullet}OH$ [25].

We have shown that in intact brain mitochondria, difenoconazole increases the rate of H_2O_2 production by 46% (Fig. 6). Overproduction of H_2O_2 probably occurs in all parts of the brain, but their sensitivity to damage is determined by the population of neurons. Almost 75% of dopaminergic neurons are located in the ventral midbrain [26]. The preferential accumulation of labile iron in the midbrain [27] promotes the formation of hydroxyl radicals in the Fenton reaction, which enhances mtDNA damage, since the ability of $^{\bullet}OH$ to damage DNA is several times higher than that of H_2O_2 [28].

None of the compounds studied reduced the amount of mtDNA damage after addition to isolated mitochondrial preparations incubated with difenoconazole. However, *in vivo*, 2,4-DNP showed the highest protective effect in the midbrain, reducing the amount of difenoconazole-induced mtDNA damage by 21% ($p<0.01$) (Fig. 7).

In addition, 2,4-DNP reduced the amount of mtDNA damage in the lungs by 16% (difference was not statistically significant, $p=0.08$). It was previously shown that 2,4-DNP effectively reduced the level of oxidative stress in the brain, prevented neuronal damage and apoptosis in Parkinson's disease models induced by rotenone [29] and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [30]. The protective ability of 2,4-DNP is associated with its ability to uncouple oxidative phosphorylation followed by an increase in the mitochondrial respiration rate, a decrease in O_2^- production, a decrease in NADH levels thus preventing the formation of ROS by mitochondrial flavoproteins [31]. On the contrary, difenoconazole inhibits ETC complex I [32], which should lead to increased NADH levels and ROS hyperproduction (Fig. 8). We have shown that the addition of 2,4-DNP to intact mitochondria increases the respiration rate from 61.01±5.3 nmol/min/mg protein to 79.16±8.2 nmol/min/mg protein (Fig. 9), and also reduces the difenoconazole-induced increase in the rate of H_2O_2 production to 47.80±8.7 pmol/min/mg protein ($p<0.01$), to the level of control mitochondria (Fig. 6).

Although 2,4-DNP has previously been shown to be effective in the treatment of Alzheimer's disease [33], Parkinson's disease [34], Huntington's disease [35], focal cerebral ischemia [36], and traumatic brain injury [37], it is unlikely that in the near future it will be introduced into clinical practice. Nevertheless, our study demonstrated its effectiveness in protecting mtDNA during difenoconazole toxicity (Fig. 4).

In this regard, MB is more attractive than 2,4-DNP. MB is currently approved for the treatment of methemoglobinemia (trade name PROVAYBLUE®) (NCT03446001, 2018) and it is in clinical trials for the treatment of Alzheimer's disease. MB functions

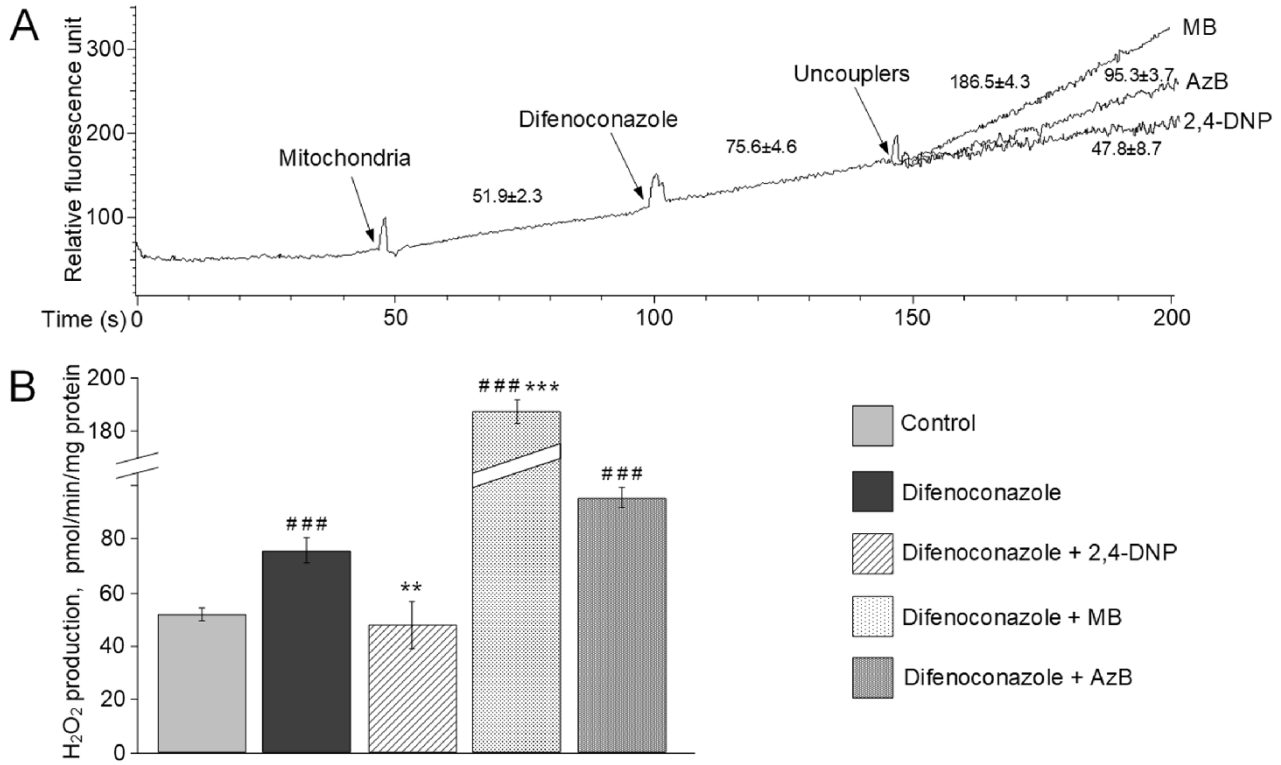

Figure 6. Effect of difenoconazole and uncouplers on the rate of H_2O_2 formation. (**A**) A curve of H_2O_2 accumulation after adding each of the test compounds. (**B**) The rate of H_2O_2 release. All measurements were carried out at least in triplicate. ** p<0.01, *** p<0.001 – differences are statistically significant as compared to mitochondria treated with difenoconazole. ### p<0.001 – differences are statistically significant as compared to control mitochondria.

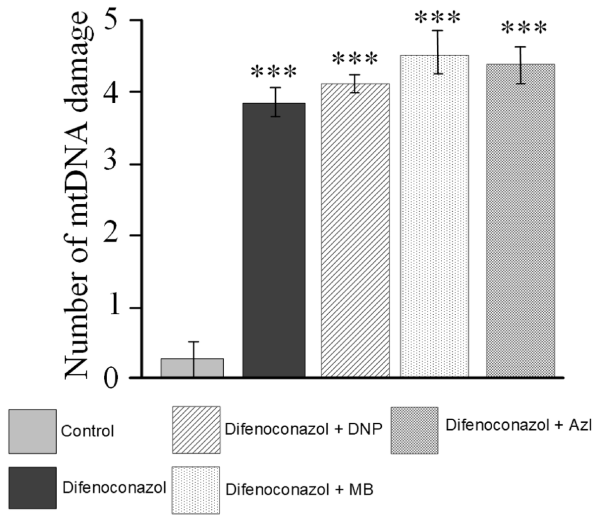

Figure 7. The i*n vitro* effect of difenoconozole and uncouplers on the amount of mtDNA damage in midbrain mitochondria. Concentrations of the test substances were: 20 μM difenoconazole, 1 mM 2,4-DNP, 1 μM MB, 1 μM AzI. All measurements were performed for at least six mtDNA fragments. *** p<0.001 – differences are statistically significant as compared to control mitochondria.

as an alternative electron carrier, bypassing complex I of the ETC (Fig. 8). In addition, MB can activate antioxidant, anti-apoptotic, and anti-inflammatory signaling pathways, inhibit monoamine oxidase and NO synthase, and thus has a neuroprotective effect in a number of diseases [38]. However, we showed that MB had no effect on reducing the amount of mtDNA damage (Fig. 3, 4) and improving memory (Fig. 1). It can be assumed that the reason for the lack of a protective effect of MB is associated with its pro-oxidant properties. MB can bypass inhibited complex I, but this process is associated with increased H_2O_2 production [39]. We have shown that MB causes a 2.5-fold increase in the rate of H_2O_2 production as compared to brain mitochondria treated with difenoconazole alone (Fig. 6). It is possible that an increase in the production of low concentrations of H_2O_2 associated with MB may trigger a signaling pathway that protects cellular components, including mitochondria [40]. However, under conditions of "severe oxidative stress", when difenoconazole caused a sharp increase in the rate of ROS production, the compensatory reactions induced by MB could not provide a protective effect.

AzI, like MB, can be used as an antidote for cyanide poisoning even in low concentrations [41]. AzI is more effective than MB in inhibiting the proinflammatory cytokine tumor necrosis factor alpha (TNF-α) and monoamine oxidase A (MAO-A), which makes it an extremely promising antidepressant [42]. Indeed, we have shown that AzI reduced difenoconazole-induced mtDNA damage in the testes by 31% (p<0.001) (Fig. 2A) and in the midbrain by 14% (p=0.058) (Fig. 2B). In addition, AzI prevented the cognitive deterioration in mice treated with difenoconazole

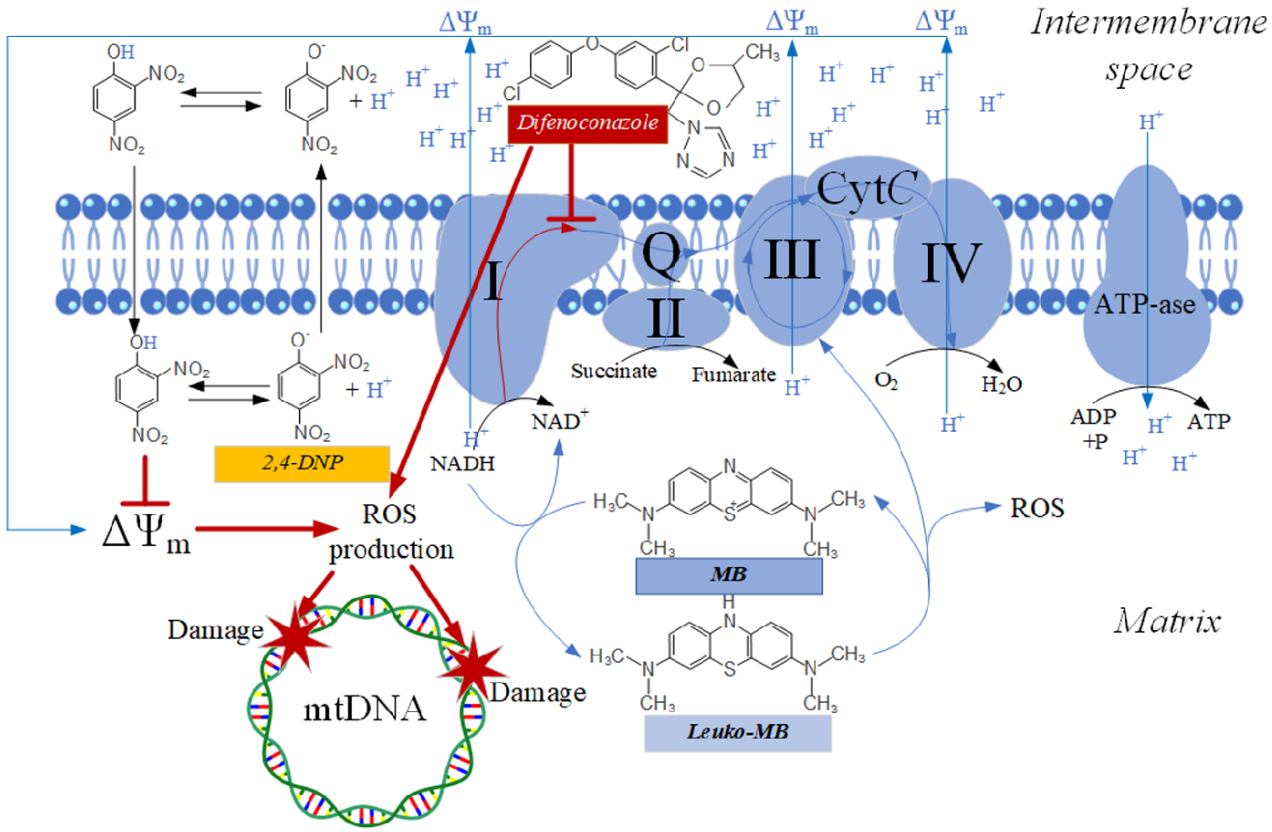

Figure 8. The mechanism of action of difenoconazole, 2,4-DNP and MB in cells.

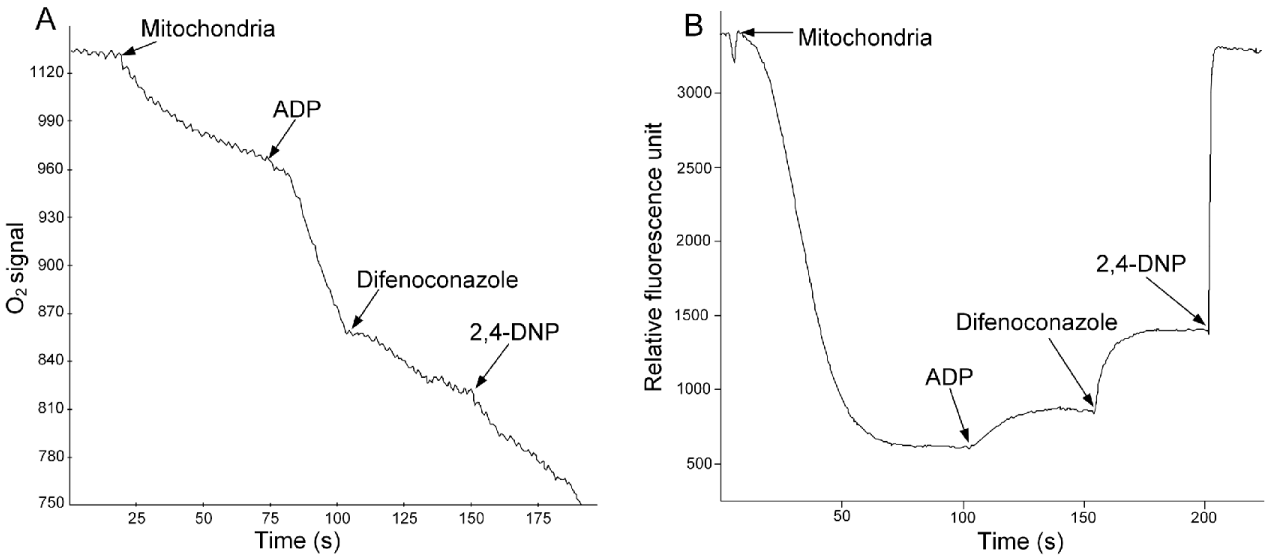

Figure 9. Bioenergetic parameters of intact brain mitochondria. (**A**) O_2 consumption rate, (**B**) Safranin O fluorescence. The following additions were made: 20 μM difenoconazole, 2 mM ADP, 1 μM 2,4-DNP, 2 μM safranin O.

(Fig. 1). Thus, it appears that AzI is more effective than MB as a pharmacological agent for reducing difenoconazole toxicity. One reason for higher effectiveness of AzI over MB may be that the former (AzI) is neutral in its quinone imine form and thus it may have faster/better diffusion across cellular and mitochondrial membranes than MB, which is positively charged [43].

CONCLUSIONS

In summary, we have demonstrated that administration of difenoconazole causes acute toxicity, manifested by a significant increase in mtDNA damage. This effect is most pronounced in the lungs, testes, heart, and midbrain. At the same time, other brain regions are damaged

to a much lesser extent. Pharmacological uncoupling of oxidative phosphorylation may protect mtDNA from difenoconazole-induced damage and prevent cognitive decline.

FUNDING

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COMPLIANCE WITH ETHICAL STANDARDS

Animals were kept and killed in accordance with the rules established by the Committee for the Care and Use of Animals of Voronezh State University, which correspond to the European Union directive 2010/63/EU regarding experiments using animals.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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ИЗУЧЕНИЕ ПРОТЕКТОРНОГО ЭФФЕКТА МИТОХОНДРИАЛЬНЫХ РАЗОБЩИТЕЛЕЙ ПРИ ОСТРОЙ ТОКСИЧНОСТИ ФУНГИЦИДА ДИФЕНОКОНАЗОЛА В РАЗЛИЧНЫХ ОРГАНАХ МЫШЕЙ

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Пестициды представляет собой серьёзную проблему для работников сельского хозяйства из-за их нейротоксического действия. Цель данного исследования — оценить способность фармакологических разобщителей окислительного фосфорилирования снижать эффект дифеноконазола на митохондриальную ДНК (мтДНК) различных органов у мышей. Инъекции дифеноконазола вызывали когнитивные дефициты у мышей, а протонофор 2,4-динитрофенол (2,4-ДНФ) и азур I (АзI) — деметилированный метаболит метиленового синего (МС) — предотвращали ухудшение когнитивных способностей у мышей при введении дифеноконазола. Дифеноконазол увеличивает скорость продукции активных форм кислорода (АФК), вероятно, за счёт ингибирования комплекса I дыхательной цепи митохондрий. мтДНК лёгких, семенников и среднего мозга наиболее чувствительна к накоплению повреждений после внутрибрюшинного введения дифеноконазола. Напротив, кора головного мозга и гиппокамп не устойчивы к действию дифеноконазола. Протонофор 2,4-динитрофенол (2,4-ДНФ) снижал скорость образования АФК и значительно сокращал количество повреждений мтДНК, вызванных дифеноконазолом в среднем мозге, а также, частично, в лёгких и семенниках. МС — альтернативный переносчик электронов, способный обходить ингибированный комплекс I, — не оказывал влияния на эффект дифеноконазола на мтДНК, тогда как его метаболит АзI — деметилированный метаболит МС — способен защищать мтДНК среднего мозга и семенников. Таким образом, митохондриально-направленная терапия является перспективным подходом к снижению токсичности пестицидов для работников сельского хозяйства.

Полный текст статьи на русском языке доступен на сайте журнала (http://pbmc.ibmc.msk.ru).

Ключевые слова: дифеноконазол; метиленовый синий; 2,4-динитрофенол; азур I; митохондриальная ДНК; разобщённое дыхание

Финансирование. Изучение когнитивных функций животных и целостности мтДНК было выполнено при поддержке гранта РНФ №22-74-00115, изучение биоэнергетических характеристик митохондрий было выполнено при финансовой поддержке Министерства науки и Высшего образования Российской Федерации в рамках Государственного задания университетам в области научной деятельности (проект № FZGU-2023-0009).

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