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Biochemistry

Experimental Study of Antimutagenic and Anticytotoxic Effects of Biorag and α-Tocopherol

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Abstract. Environmental pollution caused by human industrial and agricultural activities comes back to them in a way to harm their genetic apparatus manifested as not only hereditary diseases, but also inherited abnormalities, malignant tumors, cardiovascular, digestive, nervous systems diseases, etc. Environmental pollution factors (pesticides, heavy metals, fertilizers, carcinogens, viruses, etc.) have mutagenic and toxic effects. It is important to identify and use effective antimutagens, which can minimize the frequency of spontaneous and induced mutations. Antimutagenic and antitoxic effects of biorag and α-tocoferol are studied on adult laboratory mice (without line) for mutations induced by Bordeaux liquid and chlorofos. Oral introduction of pesticides (doze 1/2 LD50) to animals induced strong increase ($P < 0.001$) in chromosomal aberrations (multiple fragmentation, lyses), genomic mutations (triploidy, tetraploidy), Pathological mitosis (K-mitosis, hollow metaphase, adhesion of chromosomes) and destruction of hollow nucleus. Chromosome preparations from animals' bone marrow cells were prepared in accordance with the Ford and Woollam methods. All numerical data were processed with various statistical method, and t-criteria was determined with Student's t-distribution. biorag and α-tocoferol have greatly expressed antimutagenic and anticytotoxic effect and statistically reliably reduce mutagenic and cytotoxic effect of chlorofos and Bordeaux liquid. At separate effect of Bordeaux liquid and chlorofos (dose 1/2 LD50) the frequency of chromosomal anomalies was 12.5 % and 8.78%, pathologic mitosis – 14.8 % and 14.6%, and hollow nucleus – 4.8% and 2.45%. After addition of biorag and α-tocoferol these indices decrease accordingly, to 4.8 % and 2%; 5.7% and 5.2; 1.6% and 1.43 % ($p < 0{,}001$). As experiments show, application of biorag and α-tocoferol for medical purpose is prospective, especially for people in contact with harmful mutagenic substances or poisoned with cancerogens. © *2026 Bull. Natl. Acad. Sci. Georg.*

Keywords: mutagen, chromosomal mutation, biorag, α-tocoferol

Introduction

Environmental pollution, primarily caused by human economic activity, acts as a damaging factor for living organisms. This impact is not limited to the induction of malignant neoplasms and hereditary diseases but also contributes to the development of cardiovascular, neurological, gastrointestinal, and other pathologies (Mnif et al., 2011; Sande et al., 2011; DeMarini, 2020).

In agriculture, the extensive use of pesticides and their large-scale accumulation in soil, water, and the atmosphere trigger a series of ecological changes that exert harmful effects both on various organisms and on human health, including that of

future generations. Consequently, the development of preventive measures aimed at reducing the adverse biological effects of these substances is of paramount importance (Sande et al., 2011). One promising approach is the application of effective antimutagens, which are capable of minimizing the frequency of both spontaneous and induced mutations (Alston, 2016; Zhu et al., 2022).

The objective of the present study was to determine the mutagenic and cytotoxic activity of the pesticides Bordeaux mixture and chlorophos, as well as to evaluate the antimutagenic and anticytotoxic potential of biorag and vitamin α-tocopherol (Bartolini et al., 2021). For this purpose, the antimutagenic and anticytotoxic effects of the bioenergetic activator biorag and vitamin α-tocopherol were investigated in laboratory white mice under conditions of mutation induction by the pesticides Bordeaux mixture and chlorophos (Gakhokidze et al., 2010).

Materials and Methods

The experiments were conducted on white mice (without line). The pesticides selected for investigation were the widely used Bordeaux mixture and chlorophos. These substances were administered to the mice orally via a gastric probe at doses corresponding to ½ LD_{50}. Chromosome preparations were obtained using the Ford and Woollam method (Ford et al. 1963). Structural chromosomal aberrations and genomic mutations were recorded according to the method of N. Dubinin, while the analysis of pathological mitoses was performed in accordance with the classification proposed by I. Alov. Disturbed interphase nuclei were assessed by a method modified by A. Pirtskhelani (Dubinin and Pashin, 1978; Alov, 1972; Pirtskhelani and Mamaladze, 1991).

Results and Discussion

The disturbances induced by the pesticides Bordeaux mixture and chlorophos in laboratory mice, as well as the obtained results, are presented in the table. It was observed that in the case of Bordeaux mixture administration, the frequency of structural chromosomal aberrations and genomic mutations reached 12.5%, pathological mitoses 14.8%, and interphase nuclear disturbances 4.8%. In the control group, the corresponding values were 0.1%, 3.7%, and 1.1%, respectively. Structural chromosomal aberrations were less pronounced than pathological mitoses.

Of structural chromosomal aberrations, chromosomal lysis occurred more frequently than single or multiple fragments. As for genomic mutations, the formation of triploid cells was most commonly observed; with regard to pathological mitoses, hollow metaphases and chromosome stickiness

Table. Experimental study of the antimutagenic and anticytotoxic effects of biorag and α-tocopherol under exposure to chlorophos and Bordeaux mixture (pesticide dose: ½ LD_{50}; antimutagen dose: therapeutic range)

Options	Number of animals	Number of examined metaphases	Structural chromosomal aberrations, %	Pathological mitoses (%)	Hollow nuclei (%)
Bordeaux mixture	5	500	12.5±1.3	14.8±0.6	4.8±0.6
Bordeaux mixture + α-tocopherol	5	450	4.8±0.9	5.7±1.1	1.6±0.3
Bordeaux mixture + biorag	5	430	3.57±0.8	4.22±1.2	1.2±0.2
Bordeaux mixture+ α-tocopherol + biorag	5	500	2.7±0.6	4.0±1.3	1.1±0.1
Chlorophos	5	400	8.78±1.7	14.6±1.6	2.45±0.5
Chlorophos + biorag	5	400	2.0±0.7	5.2±1.1	1.43±0.3
Control/reference	5	500	1.0±0.2	3.7±0.8	1.1±0.3

were predominant, whereas C-metaphases and tripolar metaphases were less frequently recorded.

I. Alov proposed a classification of pathological mitoses that describes not only their morphological features but also the cytophysiological mechanisms underlying their formation (Alov, 1972). According to this classification, the pathological mitoses observed in our study – chromosome stickiness, lysis, and fragmentation – should be attributed to the direct action of the agent on the chromosomes, resulting in their damage. In contrast, the emergence of hollow metaphases and C-metaphases appears to be linked to the agent's interference with the mitotic apparatus, which initially leads to its disruption and subsequently gives rise to these forms of pathological mitoses.

It is noteworthy that the pesticides investigated often induced severe chromosomal abnormalities (pathological mitoses, chromosome lysis, polyploid cells). Consequently, it must be considered that cells with profound alterations are eliminated, indicating the presence of cytotoxic and, accordingly, general toxic effects.

In our earlier studies, it was established that pesticides such as Keimi, Chlopho-50, Phosphamide, Cuprousan, and others frequently induce severe chromosomal aberrations, whereas Ragil and Keltan are characterized by comparatively lower mutagenic and toxic activity. It can therefore be assumed with high probability that the general toxic effects of pesticides are primarily mediated through their mutagenic and cytotoxic properties.

Thus, Bordeaux mixture and chlorophos are distinguished by pronounced mutagenic and toxic activity (see Table). This highlights the necessity of developing preventive measures aimed at mitigating their genetic consequences. Such measures may incorporate: 1) Identification of highly hazardous mutagens and their withdrawal from use, or replacement with less harmful analogues, 2) Determination of biologically safe doses of harmful chemical agents, since higher doses are associated with adverse effects on the organism, and 3) Selection and application of effective antimutagens capable of minimizing the frequency of both spontaneous and induced mutations. The antioxidant effect of α-tocopherol is well known (Rimbach et al., 2010; Alba et al., 2008; Tucker and Townsend, 2005). In the experiment, the antimutagens biorag and α-tocopherol were employed. The table shows that under exposure to Bordeaux mixture alone, the frequency of chromosomal anomalies (hollow metaphases, C-mitoses, chromosome swelling, stickiness and/or despiralization) reached 12.5%, pathological mitoses 14.8%, and disturbed interphase nuclei 4.8%. When Bordeaux mixture was administered against the background of biorag, the pesticide-induced cytogenetic alterations decreased to 3.57%, 4.22%, and 1.2%, respectively. Against the background of α-tocopherol, these disturbances decreased to 4.8%, 5.7%, and 1.6%, respectively.

When biorag and α-tocopherol were administered jointly to animals, the cytogenetic effects induced by Bordeaux mixture decreased even further, reaching 2.7%, 4.0%, and 1.1%, respectively (see Table).

Thus, biorag demonstrated a pronounced antimutagenic and anticytotoxic effect. On average, it reduced Bordeaux mixture–induced cytogenetic disturbances by 3.5-fold or more, whereas α-tocopherol decreased them by an average of 2.6-fold.

The antimutagenic and anticytotoxic activity was mostly pronounced under the combined influence of α-tocopherol and biorag, where Bordeaux mixture–induced chromosomal structural aberrations decreased to 2.7%, pathological mitoses to 4.0%, and hollow nuclei to 1.1%.

In subsequent series of experiments, the effect of biorag on the mutagenic and cytotoxic activity of the pesticide chlorophos was investigated.

The results showed that when animals in advance received subcutaneous injections of biorag (therapeutic dose) for five consecutive days, followed on the fifth day by oral administration of chlorophos (dose: ½ LD_{50}), the mutagenic and cytotoxic

effects were markedly reduced (see Table). Specifically, if under chlorophos exposure alone, the frequency of chromosomal structural aberrations reached 8.78%, pathological mitoses 14.6%, and hollow nuclei 2.45%, with biorag, chlorophos-induced cytogenetic alterations decreased to 2.0%, 5.2%, and 1.4%, respectively. In the control group, the corresponding values were 1.0%, 3.7%, and 1.1%.

Conclusion

Thus, the studies conducted demonstrate that the pesticides Bordeaux mixture and chlorophos are characterized not only by mutagenic activity, expressed through an increased frequency of chromosomal anomalies, but also by general toxic and cytotoxic effects, as evidenced by the elevated numbers of pathological mitoses and disturbed interphase nuclei. Our findings are consistent with both our previous results and those reported by other authors, in which various pesticides (Keimi, Cuprozin, Keltan, potassium permanganate), carcinogens (benzo[a]pyrene, Arnold's base), and antimutagens (interferon, Ragil, Ematon, and various multivitamins) were investigated.

ბიოქიმია

ბიორაგისა და α-ტოკოფეროლის ანტიმუტაგენური და ანტიციტოტოქსიკური მოქმედების შესწავლა ექსპერიმენტში

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გარემოს დაბინძურება, რომელიც ძირითადად, ადამიანის სამრეწველო და სასოფლო-სამეურნეო საქმიანობითაა გამოწვეული, ორგანიზმის გენეტიკური აპარატის დამაზიანებელი ფაქტორების სახით კვლავ ადამიანს უბრუნდება, რასაც უკავშირდება არა მხოლოდ მემკვიდრული დაავადებების, არამედ ავთვისებიანი სიმსივნეებისა და ისეთი პათოლოგიების წარმოშობა, როგორებიცაა გულ-სისხლძარღვთა, საჭმლის მომნელებელი, ნერვული სისტემისა და სხვა დაავადებები. გარემოს დამბინძურებლები (პესტიციდები, მძიმე ლითონები, სასუქები, კანცეროგენები, ვირუსები და სხვ.) მუტაგენური და ტოქსიკური მოქმედებით ხასიათდება. ამდენად, მნიშვნელოვანია ეფექტური ანტიმუტაგენების განსაზღვრა და გამოყენება, რომლებიც მინიმუმამდე შეამცირებს ბუნებრივი და ინდუცირებული მუტაციების სიხშირეს.

ბიორაგისა და α-ტოკოფეროლის ანტიმუტაგენური და ანტიტოქსიკური ეფექტი შესწავლილ იქნა ლაბორატორიულად ზრდასრული უხაზო თეთრი თაგვების ორგანიზმზე, პესტიციდებით – ბორდოს სითხისა და ქლოროფოსით მუტაციების ინდუქციის შემთხვევაში. ცხოველების ორგანიზმში პესტიციდების პერორალურად შეყვანა (დოზა: ½. LD50) ქრომოსომათა სტრუქტურული დარღვევების (მრავალჯერადი ფრაგმენტაცია, ლიზისი), გენომური მუტაციების (ტრიპლოიდი, ტეტრაპლოიდი), პათოლოგიური მიტოზის (K-მიტოზი, ღრუ მეტაფაზა, ქრომოსომების შეწებება) ძლიერ ზრდასა (P<0,001) და ღრუ ბირთვების რღვევას იწვევდა. ცხოველების ძვლის ტვინის უჯრედების ქრომოსომული პრეპარატები მომზადდა ფორდისა და ვულამის მეთოდით. ყველა რიცხვითი მონაცემი დამუშავდა სხვადასხვა სტატისტიკური მეთოდის გამოყენებით, t კრიტერიუმი კი განისაზღვრა სტიუდენტის განაწილებით. ბიორაგი და α-ტოკოფეროლი გამოხატული ანტიმუტაგენური და ანტიციტოტოქსიკური მოქმედებით ხასიათდება და სტატისტიკურად სარწმუნოდ ამცირებს ქლოროფოსისა და ბორდოს ხსნარის მუტაგენურ და ციტოტოქსიკურ მოქმედებას. ბორდოს ხსნარისა და ქლოროფოსის ცალ-ცალკე მოქმედების შემთხვევაში (დოზა ½. LD_{50}) ქრომოსომული ანომალიების სიხშირე შესაბამისად, იყო 12,5% და 8,78%, პათოლოგიური მიტოზების – 14,8% და 14,6%, ღრუ ბირთვებისა კი – 4,8% და 2,45%. ბიორაგისა და α-ტოკოფეროლის დამატების შედეგად აღნიშნული მაჩვენებლები შესაბამისად, შემცირდა 4,8% და 2%-მდე; 5,7% და 5,2%-მდე; 1,6% და 1,43%-მდე (p<0,001). ჩატარებული ექსპერიმენტების შედეგად შეიძლება დავასკვნათ, რომ ბიორაგისა და α-ტოკოფეროლის გამოყენება სამედიცინო პრაქტიკაში პერსპექტიულია, განსაკუთრებით მათთვის, ვისაც შეხება აქვს მავნე მუტაგენურ ნივთიერებებთან, ასევე კანცეროგენებით მოწამლული პაციენტებისთვის.

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