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A CYTOGENETIC STUDY OF THE ANTIMUTAGENIC POTENTIAL OF HERBAL INFUSIONS FROM *MATRICARIA CHAMOMILLA* L. AND ACHILLEA MILLEFOLIUM L. (FAM. *ASTERACEAE*)

Abstract. Due to increase of hazardous factors in the environment, it becomes relevant to search for effective protectors of natural origin for the correction of toxic and genetic effects induced by xenobiotics. Using the test for count chromosomal abnormalities in cells of root germinal meristem of barley seeds, the mutagenic and antimutagenic activity of infusions from chamomile (Matricaria chamomilla) and yarrow (Achillea millefolium) were studied. The studied infusions with various preparation methods (concentrated, diluted and phyto tea) did not show mutagenic activity. The frequency of structural mutations was at the level of negative control (distilled water). The ability of yarrow and chamomile infusions to reduce MMS-induced mutagenesis has been established. For direct and reverse treatment of seeds with diluted infusions or herbal tea of medicinal plants and mutagen methyl methanesulfonate (MMS, positive control) a statistically significant decrease of MMS-induced was observed ($p < 0.05$). Moreover, the level of inhibition of the mutation process depended on the sequence of exposure to infusions and mutagen, as well as the type of infusion. The effectiveness of the antimutagenic effect of the studied infusions was evaluated by the reduction factor. The reduction factor in the infusion of diluted chamomile and chamomile tea was 67.0% and 62.0%, respectively, which indicates the ability to inhibit MMS-induced mutagenesis by more than 60% from Matricaria chamomilla infusions. The results indicate a strong antimutagenic effect of diluted infusions of chamomile. The magnitude of the reduction factor in yarrow infusions indicates the ability of infusions from Achillea millefolium to inhibit MMS-induced mutagenesis by 40-50% with preliminary exposure to barley to MMS. The reduction factor subsequent to the effect of infusions after MMS was 45-50%. The results obtained indicate the presence of antimutagenic activity in infusions of chamomile and yarrow, due to the presence of biologically active substances of various nature in plants of these species.

Key words: biologically active substances, medicinal plants, mutagenesis, antimutagenic activity, chromosomal aberrations.

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Asteraceae тұқымдас *Matricaria chamomilla* L. және *Achillea millefolium* L. дәрілік өсімдіктердің тұнбаларының антимутагендік потенциалын цитогенетикалық зерттеуі

Аңдатпа. Қоршаған ортадағы экологиялық қауіпті факторлардың көбеюіне байланысты ксенобиотиктер тудырған уытты және генетикалық әсерлерді түзету мәселесі маңызды болып табылады. Бұл мәселенің шешу жолдарының бірі – табиғи шығу тегі тиімді протекторларды іздеу болып табылады. Арпа тұқымының тамыр гермиалды меристемасының жасушаларында хромосомалық абберациялар санының есепке тестті пайдалана отырып, түймедақ (Matricaria chamomilla) және кәдімгі мыңжапырақ (Achillea millefolium) тұнбаларының мутагендік және антимутагендік белсенділігі зерттелді. Әр түрлі дайындау әдістерімен зерттелген тұнбалар (концентрацияланған, сұйылтылған және фито-шай) мутагендік белсенділікті көрсетпеді. Мутациялардың жиілігі теріс бақылау (су) деңгейінде болды. Түймедақ пен мыңжапырақ тұнбалардың индукцияланған мутагенезді төмендету бағытында өзгерту мүмкіндігі анықталды.

Дәрілік өсімдіктердің сұйылтылған тұнбасы мен фито-шайы мутаген метил метансульфонатпен (ММС, позитивті бақылау) бірге тұқымдарды тікелей және кері өңдеу кезінде ММС қоздырылған мутагенез деңгейінің статистикалық маңызды төмендеуі байқалды ($p < 0.05$). Сонымен қатар, мутация процесінің тежелу деңгейі тұнба мен мутагеннің әсер ету тәртібіне, сондай-ақ тұнба түріне байланысты болды. Зерттелетін тұнба антимутагендік әсерінің деңгейі редукциялық фактор бойынша бағаланды. Түймедақтың сұйылтылған тұнбасы мен түймедақ шайының редукциялық факторы 67,0% және 62,0% құрады, бұл Matricaria chamomilla тұнбасының ММС қоздырған мутагенезді 60%-дан астам ингибирлеу қабілеттілігін көрсетеді. Нәтижелер түймедақ сұйылтылған тұнбасы беретін мықты антимутагендік әсерді көрсетеді. Тұқымдарды ММС-тен бұрын кәдімгі мыңжапырақ тұнбасымен өңдеу экспериментінде редукциялық факторының мөлшері Achillea millefolium ММС индукцияланған мутагенезді 40-50% төмендету қабілеттілігін көрсетті. Тұқымдарды MMS-тен кейін мыңжапырақ тұнбасымен өңдеу экспериментінде редукциялық факторы 45-50% құрады. Алынған нәтижелер түймедақ және жусан өсімдіктердің құрамындағы әртүрлі биологиялық белсенді заттардың болуына байланысты зерттелген тұнбалар антимутагендік белсенділігі бар екенін көрсетеді.

Түйін сөздер: биологиялық белсенді заттар, дәрілік өсімдіктер, мутагенез, антимутагендік белсенділік, хромосомалық аберрациялар.

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Цитогенетическое исследование антимутагенного потенциала настоев лекарственных растений *Matricaria chamomilla* L. и *Achillea millefolium* L. семейства *Asteraceae*

Аннотация. В связи с увеличением экологически опасных факторов в окружающей среде становится актуальным поиск эффективных протекторов природного происхождения для коррекции токсических и генетических эффектов, индуцируемых ксенобиотиками. С помощью теста по учету хромосомных аберраций в клетках корневой зародышевой меристемы семян ячменя были изучены мутагенная и антимутагенная активности настоев ромашки аптечной (Matricaria chamomilla) и тысячелистника обыкновенного (Achillea millefolium). Исследуемые настои при различных способах приготовления (концентрированный, разбавленный и фито-чай) не проявили мутагенной активности. Частота структурных мутаций была на уровне негативного контроля (вода). Установлена способность настоев ромашки аптечной и тысячелистника обыкновенного модифицировать индуцированный мутагенез в сторону его снижения. При совместной прямой и обратной обработке семян разбавленными настоями и фито-чаем лекарственных растений и мутагеном метилметансульфонатом (ММС, положительный контроль) наблюдалось статистически значимое снижение уровня ММС-индуцированного мутагенеза ($p < 0,05$). При этом уровень ингибирования мутационного процесса зависел от последовательности воздействия настоев и мутагена, а также вида настоя. Эффективность антимутагенного действия изучаемых настоев оценивали по редукционному фактору. Редукционный фактор у настоя разбавленного ромашки и чая ромашки составил соответственно 67,0% и 62,0%, что свидетельствует о способности ингибировать настоями из Matricaria chamomilla ММС-индуцированный мутагенез более чем на 60%. Полученные результаты указывают на сильный антимутагенный эффект, дающий разбавленными настоями ромашки аптечной. Величина редукционного фактора у тысячелистника обыкновенного свидетельствует о способности настоев из Achillea millefolium ингибировать ММС-индуцированный мутагенез на 40-50% при предварительном до ММС воздействии на семена ячменя. Редукционный фактор при последующим после ММС воздействии настоев составил 45-50%. Полученные результаты свидетельствуют о наличии антимутагенной активности у настоев ромашки аптечной и тысячелистника обыкновенного, обусловленной наличием биологически активных веществ различной природы в растениях этих видов.

Ключевые слова: биологически активные вещества, лекарственные растения, мутагенез, антимутагенная активность, хромосомные аберрации.

Abbreviations: MMS – methyl methanesulfonate

Introduction

Large-scale environmental pollution by potential mutagens and genotoxicants poses a serious threat to biological diversity, including humans, which has been formed during evolution over a huge period of time [1-4]. Therefore, it becomes relevant to search and create means for protecting the body from the negative effects of hazardous factors in the environment. Biologically active substances of natural origin are promising in this regard, because of their capability of increasing the body's immune status, activating reparation systems, and intercepting free radicals, in particular reactive oxygen species. One of the promising sources of such biologically active substances are medicinal plants [5-10]. Among the great variety of flora of Kazakhstan, which has about 6,000 species of higher plants, at least 500 species are medicinal plants [11-13].

One of the largest families among flowering plants is the *Asteraceae* family (*Compositae*), which has more than 23,000 species that grow on all continents. This family includes varieties of valuable medicinal plants, which leads to the growing popularity of herbal medicine based on them. A number of species of medicinal plants from the *Asteracea* family are used as pain medications, disinfectants, antipyretic and anti-inflammatory drugs [14].

The genus *Matricaria* of the *Asteracea* family has about 25 species and it of great interest for research in this area, due to the high content of various biologically active substances with antimutagenic activity. The most common and used in traditional medicine is chamomile. It contains essential oil (chamomile oil); derivatives of glycosides of apigenin, luteolin, quercetin; coumarins; free organic acids, including caprylic, antemisic, isovalerianic, salicylic); polysaccharides, tannins, vitamins (nicotine and ascorbic), etc. Chamomile flowers contain a large amount of flavonoids [11].

Another perspective source of biologically active substances with antimutagenic effect is yarrow (*Achillea millefolium* L.) from the genus *Achillea*, Asteraceae family (*Compositae*). Yarrow is widely used in traditional medicine as a medicinal plant. This type of plant contains vitamin K, carotene, ascorbic acid, alkaloid, achillein (0.05%), sesquiterpenes, tannins, resin, up to 1% essential oil, which contains up to 30% azulenes, pinene, borneol, up to 13% esters, camphor, thujone, up to 10% cineol, formic, acetic and isovaleric acid, up to 20% alcohols. The genus *Yarrow* has about 150 species.

Screening the medicinal flora of Trans-Ili Alatau for antimutagenic and gene-protective activity, as well as studying the mechanisms of action of biologically active substances contained in them at the cellular and molecular level, is relevant and promising. The prospect of the study lays in the possibility of recommending certain types of plants to create collections with antimutagenic activity, using of it will reduce the risks of hereditary and oncological diseases. The conducted studies will expand the spectrum of action of known medicinal plants with another type of activity – antimutagenic.

The purpose of this study was to study the antimutagenic activity of infusions of medicinal chamomile plants of *Matricaria chamomilla* L. (*Asteraceae*) and *Achillea millefolium* L. (*Asteraceae*). Analysis of the genetic activity of infusions of the studied species of medicinal plants was carried out in two stages. At the first stage, the mutagenic activity of infusions of different concentrations was studied with the aim of selecting options that would not give a mutagenic effect, and at the second stage, the tread ability of the selected infusions was studied with barley seeds acting together with the mutagen.

Materials and methods

In experimental cytogenetic studies, barley seeds (*Hordeum vulgare* L.) of the Baysheshek strain, zoned in Almaty region, were used as an object for studying the antimutagenic potential of chamomile and yarrow infusions. The seeds of barley (*Hordeum vulgare* L.) are widely used in cytogenetic studies as a test object. It is associated with a small number of chromosomes equal to 7 pairs (2n = 14), which differ in large sizes (6-8 microns) [15]. Mutagenic and antimutagenic activities studies were carried out with infusions of medicinal plants from the *Asteraceae* family – yarrow (*Achillea millefolium* L.) and chamomile (*Matricaria chamomilla* L.). The infusion of chamomile (*Matricaria chamomilla* L.) is prepared mainly from flowers. The infusion of chamomile (*Matricaria chamomilla* L.) is useful for the treatment of abdominal pain, irritable bowel syndrome and insomnia. It has anti-inflammatory and bactericidal action [16, 17]. Infusion of yarrow (*Achillea millefolium* L.), which most often use leaves and inflorescences, has anti-inflammatory, wound healing and anti-allergenic properties. This plant is used in traditional medicine to treat a number of diseases, in particular for the treatment of gastric ulcer, gastritis and stomatitis [18].

A negative control was the natural level of mutation in seeds germinated on distilled water, and a positive level was the level of MMS-induced mutations. The standard mutagen methyl

methanesulfonate (MMS, $C_2H_6O_3S$) was used at a concentration of 10 mg/L [20]. MMS is a direct acting alkylating agent and in standard short-term *in vivo* and *in vitro* tests exhibits mutagenic activity. In the *umu* test on the strain *S. typhimurium* TA1535/ pSK1002, it induces an SOS response; in bacteria, in the absence of metabolic activation, it induces point mutations. In addition, MMS can cause somatic and sex-linked recessive lethal mutations in *Drosophila melanogaster*. MMS induces a neoplastic transformation in rodent cell cultures, increasing the frequency of sister chromatid exchanges and chromosomal aberrations. *In vivo* methyl methanesulfonate causes mutations in the germ cells of mice, *in vitro* in human cells causes the formation of micronuclei, single-stranded breaks, unplanned DNA synthesis, gene mutations and sister chromatid exchanges. In somatic rodent cells, MMS induces chromosomal aberrations and chromatid exchanges. It is precisely the wide range of genetic activity manifested in the battery of various test systems that explains the choice of methyl methanesulfonate as a positive control as a genotoxic and mutagenic factor [19, 20].

To study the mutagenic / antimutagenic potential of infusions of medicinal plants, chamomile (*Matricaria chamomilla* L.) and yarrow (*Achillea millefolium* L.), barley seeds were preliminarily treated with the herbal infusions. Infusions were prepared according to the recipe indicated in the pharmaceutical instructions. Three types of infusions were studied for antimutagenic activity: concentrated (according to the recipe), diluted (concentrated infusion, diluted 2 times) and phyto tea.

The separate and combined effects of infusions and MMS on barley seeds were studied. Soaking seeds was carried out in each solution for 4 hours. The treated seeds were washed and germinated in Petri dishes on filter paper moistened with distilled water under thermostat conditions at t 25 ± 1^{0}C. A day later, germinated seeds with a primary root length of 0.5 cm were transferred onto filter paper moistened with an aqueous solution of 0.01% colchicine for 4 hours in order to accumulate metaphase plates. Then, the roots were fixed in alcohol-vinegar mixture (1: 1), and after 24 hours they were transferred to 70% alcohol for long-term storage [21].

The fixed material was subjected to cold hydrolysis by placing it in a dilute aqueous cooled HCl (1: 1) solution for 40-50 minutes at a temperature of 4^{0}C. As a result of weak hydrolysis of DNA, free aldehyde groups are formed that interact with the dye, and the chromosomes become fuchsia. After staining, the roots were placed in freshly prepared sulphurous water to remove dye from the cells that did not react with DNA. Next, maceration was performed using the cytase enzyme (a mixture of cellulitic enzymes of the salivary gland of the grape snail), which destroys the intercellular substance and cell walls of plant cells, facilitating the distribution of a monolayer of metaphase plates on microscope slide. The obtained preparations with metaphase plates were kept in a refrigerator for 24 hours at a temperature of -74±1^{0}C to obtain constant cytological preparations.

To determine the mutagenic / antimutagenic potential of infusions of medicinal plants, a metaphase method for analyzing chromosomal aberrations was used. The cytogenetic test widely used by researchers gives us information about the types of structural mutations and their frequency [1, 20, 22]. Metaphase plates were analyzed on an Olympus BX 43F optical microscope (Olympus, Japan). In each embodiment, 400-500 metaphases were analyzed. The effectiveness of reducing the frequency of MMS-induced chromosomal aberrations (the effectiveness of antimutagens) was determined by the value of the reduction factor (RF). With 25-40% inhibition, the antimutagenic effect was considered moderate, with more than 40% strong, and with less than 25% the antimutagenic effect was not recognized as a positive result.

Statistical analysis of the results was carried out using the program "Data Analysis" Microsoft Excel, StarPlus. In each variant, the average values and the standard errors of the means were calculated. To establish the significance of differences between the average values of the various options, Student's test was used. The differences between the data were considered statistically significant with a confidence level of 0.95.

Results and Discussion

This section presents the results of a cytogenetic study of the antimutagenic potential of various concentrations infusions of chamomile and yarrow. Analysis of the genetic activity of infusions of the studied species of medicinal plants was carried out in two stages. At the first stage, the mutagenic activity of infusions of different concentrations was studied in order to select options that would not give a mutagenic effect. At the second stage, the DNA protective ability of the selected varieties of infusions was studied with a combined action with the mutagen on barley seeds.

The study of mutagenic and antimutagenic activity of herbal infusions from chamomile (Matricaria chamomilla L.).

The results of a cytogenetic study of the cell population of the root germinal meristem of barley seeds, separately and combined with MMS and chamomile infusions, are presented in Table 1. The natural (spontaneous) mutation level in the cells of the root germinal meristem of barley seeds germinated in distilled water was 1.25%. The number of chromosomal aberrations per 100 metaphases was slightly higher and amounted to 1.46. As a result of seed treatment, methyl methanesulfonate induced structural rearrangements of chromosomes, the level of which was statistically significantly higher than the negative control. Thus, the frequency of aberrant metaphases increased from 1.25% (control) to 5.33% (MMS), and the number of structural mutations increased from 1.46 to 6.44, respectively, i.e., 4.3 times ($p < 0.001$). Moreover, the level of chromosomal type rearrangements increased 2.7 times, and the number of chromatid aberrations per 100 cells statistically significantly increased 6.7 times ($p < 0.001$).

A statistically significant increase in chromatid type structural mutations indicates a greater sensitivity of DNA to the damaging effects of the mutagen in the S phase (synthetic phase) and G_2 phase (postsynthetic phase) of the cell cycle. In the spectrum of chromosomal aberrations, various rearrangements were noted, but paired and single terminal deletions (fragments of chromosomes), paired and single interstitial deletions, centric and acentric rings, and point fragments prevailed (Figure 1). It should be noted that anaphases with various types of rearrangements were also observed with high frequency, including chromosome lagging, bridges, single fragments, and multipolar mitoses (Figure 2).

Table 1 – The frequency and spectrum of structural chromosome abnormalities induced by separate and combined treatment of barley seeds with methyl methanesulfonate and chamomile infusions

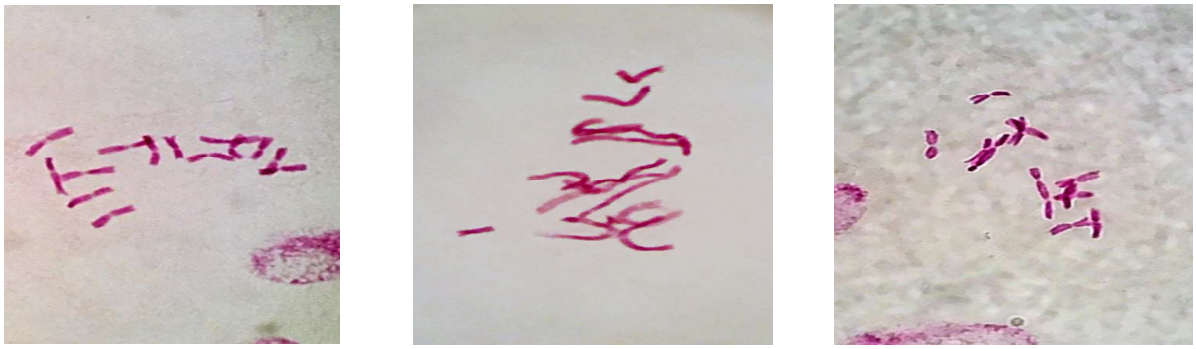
Variant	Number of studied cells	Frequency of aberrant cells (M ± m%)	Number of chromosomal aberrations per 100 metaphase cells		
			Total aberrations	chromosome type	chromatid type
Water (negative control)	480	1,25±0,51	1,46±0,55	0,83±0,41	0,63±0,36
MMS, 10 mg / L (positive control)	450	5,33±1,06*	6,44±1,16*	2,22±0,69	4,22±0,95*
Concentrated infusion	510	2,16±0,64	2,55±0,70	0,98±0,44	1,57±0,55
Diluted infusion	530	1,51±0,53	1,70±0,56	0,75±0,38	0,94±0,42
Chamomile tea	500	1,40±0,53	1,40±0,53	0,60±0,35	0,80±0,40
Concentrated infusion + MMS	530	3,96±0,85	4,34±0,89	1,89±0,59	2,45±0,67
Diluted infusion + MMS	520	1,92±0,60••	2,12±0,63••	0,96±0,43	1,15±0,47••
Chamomile tea + MMS	530	2,08±0,62••	2,45±0,67••	1,32±0,50	1,13±0,46••
MMS+ concentrated infusion	525	4,76±0,93	5,14±0,96	2,10±0,63	3,05±0,75
MMS + diluted infusion	495	2,83±0,75	3,03±0,77•	1,21±0,49	1,82±0,60•
MMS + Chamomile tea	490	2,45±0,70•	2,65±0,73••	1,02±0,45	1,63±0,57•

Note: * – $p < 0.001$ in comparison with the control; • – $p < 0.05$; •• – $p < 0.01$ in comparison with methyl methanesulfonate.

As a result of seed treatment with chamomile infusions of different concentrations (concentrated and diluted infusions, phyto tea), the level of chromosomal rearrangements in the cells of the apical part of the primary roots of barley did not statistically significantly exceed the control values. Nevertheless, seed treatment with concentrated chamomile infusion increased the induction of structural mutations by 1.7 times, but, as noted above, the difference was not statistically significant. The results obtained indicate the absence of mutagenic activity in chamomile infusions in the used concentrations.

In the next series of experiments, the ability of chamomile infusions to modify the mutagenic effect of MMS with their combined effect on barley seeds was studied. As can be seen from the results presented in Table 1, pre-treatment of seeds with concentrated chamomile infusion reduced the number of MMS-induced structural mutations

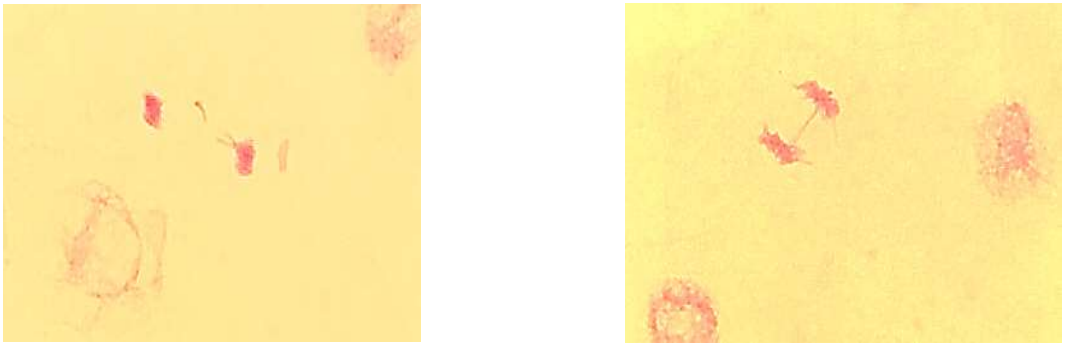
by 1.3 times, and the number of chromosome aberrations per 100 cells by 1.5 times. However, the observed decrease was not statistically significant. Pre-treatment of seeds with diluted chamomile infusion followed by mutagen treatment caused a statistically significant decrease in the level of MMS-induced mutagenesis. At the same time, the frequency of cells with chromosome aberrations decreased statistically significantly by 2.8 times ($p<0.01$), and the number of chromosomal rearrangements per 100 metaphases decreased by 3.0 times ($p<0.01$). The number of MMS-induced chromatid-type aberrations also decreased by a factor of 3.7 ($p<0.01$).



Chromosome number in normal, 2n =14 | paired terminal deletion | dicentric chromosome

Figure 1 – Structural chromosome abnormalities induced by MMS, x1000

multipolar mitoses



chromosome lag | chromosome bridge

Figure 2 – Anaphases with various types of disorders, x1000

A similar picture was observed in the variant with pre-treatment of barley with chamomile tea prior to MMS. Tea statistically significantly reduced the MMS-induced mutagenesis observed in the apical meristematic zone of primary roots. At the same time, the frequency of aberrant cells and the number of chromosomal aberrations per 100 metaphases decreased in both cases by 2.6 times ($p<0.01$). It should be noted that, as in the previous version, there was a statistically significant decrease in the level of chromatid type aberrations (3.7 times; $p <0.01$).

In the reverse combination of seed treatment (exposure to a mutagen, and then treatment with infusion), the effect of modifying the mutagenic effect of MMS was somewhat different. So, upon subsequent exposure to a concentrated chamomile infusion after a mutagen, the frequency of aberrant cells and the number of chromosome aberrations per 100 cells, the level of aberrations of the chromosome and chromatid types did not statistically significantly differ from the values of these parameters in the variant of seed treatment only with MMS.

In the variants subsequent to the treatment of seeds after MMS with diluted chamomile infusion, a statistically significant decrease in the number of chromosomal aberrations per 100 cells by 2.0 times was observed ($p<0.05$). The decrease occurred equally due to aberrations of the chromosomal and chromatid types.

Phyto tea in this sequence of seed treatment, that is, at the beginning of MMS, and then chamomile, was most effective as a protector of the mutagenic effect of MMS. The level of aberrant cells decreased by 2.2 times ($p<0.05$), and the number of chromosome aberrations per 100 metaphases – by 2.4 times ($p<0.01$). There was also a statistically significant decrease in chromatid-type aberrations ($p<0.05$).

The results obtained in this series of experiments indicate the ability of chamomile infusions to modify methyl methanesulfonate-induced mutagenesis in the direction of its reduction. Antimutagenic activity was shown in diluted infusion and phyto tea. However, the antimutagenic activity of chamomile infusions depended on the degree of their concentration. Concentrated infusion, unlike diluted infusion and tea, did not cause a statistically significant decrease in MMS-induced mutagenesis. A comparative analysis of the results of combined treatment of seeds with mutagen and chamomile infusions showed that the pre-treatment of the infusions more effectively reduces the level of induced mutagenesis than the subsequent after the mutagen.

The effectiveness of the antimutagenic effect of chamomile was evaluated by a reduction factor. The reduction factor in the diluted chamomile infusion and chamomile tea was 67.0% and 62.0%, respectively, which indicates the ability to inhibit MMS-induced mutagenesis by more than 60% from *Matricaria chamomilla* infusions. The results allow us to conclude that a strong antimutagenic effect, giving diluted infusions of chamomile containing biologically active substances.

The study of mutagenic and antimutagenic activity of herbal infusions from yarrow (Achillea millefolium L.).

Cytogenetic studies of the mutagenic/ antimutagenic potential of yarrow on barley seeds were carried out, the results of which are presented in Table 2. All types of yarrow infusions used in this series of experiments did not exhibit genetic activity. The frequency of aberrant cells and the number of chromosomal aberrations in the root meristem of barley seeds treated with infusions were at the level of negative control. At the same time, concentrated infusion increased these indicators compared to the negative control 2.0 times, however, this increase was not statistically significant. The obtained results indicate the absence of mutagenic activity in yarrow infusions in the used concentrations.

The next stage was the study of the ability of yarrow infusions containing a complex of biologically active substances to modify the mutagenic effect of MMS with the aim of revealing the antimutagenic properties of this medicinal plant. For this, barley seeds were sequentially treated first with yarrow infusions, and then with mutagen (Table 2).

As can be seen from the results presented in the Table 2, the yarrow in the combination "concentrated infusion + MMS" reduced the frequency of aberrant cells and the number of chromosome aberrations by 100 metaphases induced by MMS, but the decrease was not statistically significant compared with the positive control. As a result of pre-treatment of barley seeds with diluted infusion and tea from yarrow, the degree of inhibition of MMS-induced mutagenesis was statistically significantly increased. So, in the variant with a diluted yarrow infusion, the number of chromosomal aberrations per 100 metaphases decreased by 1.8 ($p <0.05$) compared with the processing of MMS only. The decrease in this indicator was due to structural aberrations of both the chromosomal and chromatid types. Pre-treatment of seeds with tea from yarrow

also statistically significantly reduced the yield of mutagen-induced chromosomal aberrations. In this variant, the number of chromosomal aberrations per 100 metaphases decreased by 2.1 times ($p < 0.05$). A decrease in the level of MMS-induced mutagenesis as a result of pre-treatment to tea occurred due to all types of structural aberrations of chromosomes.

Table 2 – The frequency and spectrum of structural chromosome abnormalities induced in barley seeds during separate and combined treatment with methyl methanesulfonate and yarrow infusions

Variant	Number of studied cells	Frequency of aberrant cells (M ± m%)	Number of chromosomal aberrations per 100 metaphase cells		
			Total aberrations	chromosome type	chromatid type
Water (negative control)	480	1,25±0,51	1,46±0,55	0,83±0,41	0,63±0,36
MMS, 10 mg / L (positive control)	450	5,33±1,06*	6,44±1,16*	2,22±0,69	4,22±0,95*
Concentrated infusion	480	2,50±0,71	2,71±0,74	1,04±0,79	1,67±0,58
Diluted infusion	490	1,43±0,54	1,84±0,61	0,82±0,41	1,02±0,45
Yarrow tea	510	1,76±0,58	1,76±0,58	0,78±0,39	0,98±0,44
Concentrated infusion + MMS	510	4,31±0,90	4,31±0,90	1,57±0,55	2,75±0,72
Diluted infusion + MMS	520	3,46±0,80	3,65±0,82•	1,35±0,51	2,31±0,66
Yarrow tea + MMS	510	3,14±0,77	3,14±0,77•	0,78±0,39	2,35±0,67
MMS+ concentra-ted infusion	490	5,10±0,99	5,31±1,01	1,84±0,61	3,47±0,83
MMS + diluted infusion	510	3,33±0,79	3,33±0,79•	1,18±0,48	2,16±0,64
MMS + yarrow tea	520	2,88±0,73	3,08±0,76•	1,35±0,51	1,73±0,57•

Note: * – $p < 0.001$ in comparison with the control; • – $p < 0.05$; •• – $p < 0.01$ in comparison with methyl methanesulfonate.

In the reverse combination of seed treatment (exposure to mutagen, and then yarrow treatment), the effect of modification of the yarrow with MMC-induced mutagenesis was significantly weaker and was not shown in all treatment options. So, with the treatment concentrated infusion on barley seeds after a mutagen, a tendency was observed to decrease all studied parameters, the frequency of aberrant cells, the number of chromosomal aberrations by 100 metaphases, and the frequency of aberrations of the chromosome and chromatid types. However, the observed decrease in the level of mutagenesis was statistically insignificant.

In the variants post-treatment of seeds after MMS with infusion diluted with yarrow tea, there was a statistically significant decrease in the number of chromosomal aberrations per 100 cells. At the same time, this indicator decreased by 1.9 and 2.1 times, respectively ($p < 0.05$). In the variant with post-treatment of seeds after MMS with yarrow phyto tea, the frequency of chromatid-type aberrations also statistically significantly decreased by a factor of 2.4 ($p < 0.05$).

The results obtained in this series of experiments clearly demonstrated the ability of yarrow infusions to significantly modify the level of induced mutagenesis in the direction of its decrease. It was shown that the degree of inhibition of induced mutagenesis depends not only on the concentration of infusions, but also on the sequence of exposure. The results obtained suggest that the complex of biologically active substances contained in the studied medicinal plant has antimutagenic and gene-protective activity. A comparative analysis of the results of combined treatment of seeds with mutagen and herbal infusions showed that the pre-treatment of the infusions more effectively reduces the level of induced mutagenesis than the post-treatment one.

The effectiveness of the antimutagenic effect of yarrow was evaluated by the reduction factor (RF). In pre-treatment of barley seeds, the RF in diluted yarrow infusion was 43%, and in tea – 52%. The magnitude of the reduction factor indicates the ability of infusions from *Achillea millefolium* to inhibit MMS-induced mutagenesis by 40-50% with pre-treatment to barley seeds prior to MMS. The reduction factor post-treatment of infusions after MMS was 45-50%. The results obtained indicate the presence of antimutagenic activity in yarrow infusions,

due to the presence of biologically active substances of various nature in plants of this species.

Mutagenic factors of various nature that enter our environment as a result of rapid industrialization of the agricultural sector increase the genetic risk for the population, which can result in various hereditary pathologies in newborns, malignant tumors and other diseases. One of the priority tasks of any state is genetic safety. Nevertheless, the genotoxic effect on living organisms, including humans, is almost inevitable. In this regard, the phenomenon of antimutagenesis, which is defined as a decrease in the spontaneous or induced genotoxicity of environmental mutagens, is of interest [23]. Therefore, the use of substances with antimutagenic potential is one of the main approaches to reducing the negative effect of mutagens in the environment on the human body. The use of various substances that have antimutagenic properties seems to be a possible and practicable way to prevent genotoxic effects, therefore, antimutagens can be used in the genetic safety system as protectors of the genome [24]. This work presents the results of a cytogenetic study of the antimutagenic activity of infusions of different concentrations of two species of medicinal plants from the *Asteraceae* family – chamomile (*Matricaria chamomilla* L.) and yarrow (*Achillea millefolium* L.). The antimutagenic activity of infusions from medicinal plants established in this series of experiments was shown in a significant decrease in the level of chromosomal aberrations induced by the classical mutagen methyl methanesulfonate. No statistically significant differences were found in the level of modification of the mutagenic effect of MMS with infusions of various concentrations containing biologically active substances. Also, statistically significant differences were not established in the degree of antimutagenic activity of chamomile and yarrow infusions in relation to MMS-induced mutagenesis. Currently, a fairly large number of various antimutagens have been discovered. The mechanism of action of many of them is still not fully known, and therefore they have not been widely used [25, 26]. In addition, their harmlessness to the human body has not been fully evaluated, and traditional pharmacotoxicological studies of antimutagens have not been conducted. In this regard, it is promising to search for inhibitors of induced mutagenesis among the approved drugs and medicinal plants that are widely used in traditional medicine. The most promising antimutagens capable of leveling the effect of mutagens are herbal preparations used as therapeutic agents. The antimutagenic effect of herbal preparations is due to the presence of biologically active substances in them, primarily vitamins, phenols, polyphenols, pigments, amino acids [27, 28]. These biologically active substances are present in human vegetables, fruits, berries, herbs. The genetic effects of many chemical mutagens are demonstrated through the development of oxidative stress. That is why most of the currently known antimutagens are characterized by antioxidant activity [29]. The extracts of the medicinal plants of the chamomile and yarrow studied by us, as already noted above, contain phenolic and polyphenolic compounds that can inhibit free radical processes. Some phenols are able to suppress the formation of mutagens from their precursors. Tannins, a mixture of tanning agents present in many perennials. Most tannins belong to the flavonoid class and contain a larger number of phenolic -OH groups. This allows them to firmly bind to proteins and other biopolymers. In addition, they are able to bind toxins that appear in the body, as well as salts of heavy metals. These properties of tannins can significantly reduce the induced mutability [30]. The mechanisms of action of antimutagens can be different. But most antimutagens are biologically active substances that can act on nucleic acids. It can be asserted with confidence that the antimutagenic activity of most biologically active substances of plant origin is due primarily to the ability to suppress free radical processes induced by the action of genotoxicants and to activate the work of repair systems.

Conclusion

The mutagenic and antimutagenic activity of infusions from chamomile (*Matricaria chamomilla*) and yarrow (*Achillea millefolium*) in barley seeds were studied. The studied infusions did not show mutagenic activity. At the same time, the infusions of these medicinal plants modified induced mutagenesis in the direction of its decrease. The ability of *Matricaria chamomilla* infusions to inhibit MMS-induced mutagenesis by more than 60%, and *Achillea millefolium* infusions – by 40-50% were revealed. The results obtained indicate the presence of antimutagenic activity in the studied infusions, due to the presence of various biologically active substances in plants of these species. Possible mechanisms of antimutagenic action of infusions are discussed.

Conflict of interest

All authors have read and are familiar with the contents of the article and have no conflict of interest.

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