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ANTIMUTAGENIC ACTIVITY OF ALCOHOLIC EXTRACTS OF MEDICINAL HERBS *MENTHA PIPERÍTA* L. AND *THYMUS VULGARIS* L. FAMILY *LAMIACEAE*

The global pollution of environment urges the screening of effective natural protectors for the correction of toxic and genotoxic action of xenobiotics. Medicinal herbs contain a complex of biologically active compounds that are the potential sources of such a protective agent. In current study the mutagenic and antimutagenic activity of alcoholic extracts of peppermint (*Mentha piperita* L.) and thyme (*Thymus vulgaris* L.), family *Lamiaceae,* was studied using the chromosome aberration assay (metaphase cytogenetics) in barley. The ability of extracts to significantly reduces methyl methanesulfonate-induced mutagenesis under preliminary or subsequent tinctures exposure was established ($p < 0.05$). The modifying effect of peppermint and thyme tinctures was also studied under the exposure to rocket fuel unsymmetrical dimethylhydrazine (UDMH, 1,1-dimethylhydrazine) which possess mutagenic and genotoxic activity. Subsequent test-object treatment with tinctures and UDMH significantly decrease the frequency of induced aberrant cells and number of chromosomal aberrations ($p < 0.05$). In case of both mutagens, the rate of mutagenesis inhibition depended both on the order of tinctures and mutagen treatment and extracts concentration. The efficacy of antimutagenic activity of *Mentha piperita* L. and *Thymus vulgaris* L. tinctures was assessed with reduction factor (RF) which in both experiments exceeded 40% testifying to inhibition of MMS and UDMH induced mutagenesis. Data obtained indicate the antimutagenic activity of extracts of peppermint (*Mentha piperita* L.) and thyme (*Thymus vulgaris* L.) conditioned on the presence of biologically active compounds of various nature.

Key words: medicinal herbs, biologically active compounds, induced mutagenesis, antimutagenic activity, chromosomal aberrations.

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Lamiaceae тұқымдастығының *Mentha piperíta* L. және *Thymus vulgaris* L. дәрілік өсімдіктерінің спиртті тұнбаларының антимутагенді белсенділігін зерттеу

Қоршаған ортаның экологиялық қауіпті факторлармен ғаламдық деңгейде ластануы улы және гентикалық әсерлерді түзеу үшін шығу тегі табиғи болып табылатын тиімді протекторларды іздеуді өзекті етеді. Мұндай қорғаныс құралдарының перспективті көздерінің бірі дәрілік өсімдіктердің құрамындағы биологиялық белсенді заттардың кешені болып табылады. Арпа дәндерінде хромосомалық аберрацияларды есепке алу (метафазды әдіс) тестінің көмегімен *Lamiaceae* тұқымдастығына жататын 2 өсімдік кермек жалбыз (*Mentha piperita* L.) және қарапайым жебірдің (*Thymus vulgaris* L.) спиртті тұнбаларының мутагендік және антимутагендік белсенділігі зерттелді. Жүргізілген цитогенетикалық зерттеулер нәтижесінде тұнбалардың метилметансульфонатпен (ММС) индукцияланған мутагенез деңгейін оның төмендеу жағына өзгерту қабілеті анықталды. Арпа дәндерін әртүрлі концентрациядағы спиртті өсімдік тұнбаларының ерітінділерімен және ММС (оң бақылау) классикалық мутагенімен бірлесіп тура және кері өңдеу кезінде ММС-индукцияланған мутагенез деңгейінің статистикалық маңызды төмендеуі байқалды ($p < 0,05$). Мутация процесін тежеу деңгейі тұнба мен мутагеннің әсер ету ретіне, сонымен қатар тұнба концентрациясына байланысты болды. Онымен қоса, жұмыста

қоршаған ортаның қауіпті ластаушысы, мутагендік және генотоксикалық белсенділігі бар зымыран отынының негізгі компоненті – асимметриялық диметилгидразинге (НДМГ, 1,1-ДМГ, гептил), жалбыз және жебірдің тұнбаларының модификациялық әсерлері зерттелді. ММС-пен жүргізілген тәжірибелердегідей, тест-объектіні НДМГ мен қоса жалбыз және жебірдің спирттік тұнбаларымен кезекпен өңдеу кезінде, асимметриялық диметилгидразинмен индукцияланған аберранттық жасушалар жиілігінің және хромосомалық аберрациялар санының 100 метафазаға шаққандағы ($p < 0,05$) статистикалық маңызды төмендеуі байқалды. Тежеу деңгейі зерттелетін агенттердің әсер ету ретіне және өсімдік тұнбаларының концентрациясына байланысты болды. Тұнбалардың антимутагендік әсерінің тиімділігі эксперименттің барлық нұсқаларында негізінен 40%-дан жоғары болған редукциялық фактормен (РФ) бағаланды. Бұл нәтижелер *Mentha piperita* L. және *Thymus vulgaris* L. тұнбаларының ММС және НДМГ индукцияланған мутагенезді 40%-дан астам ингибирлеу қабілетін көрсетеді. Жүргізілген зерттеулер кермек жалбыз бен қарапайым жебірдің тұнбаларында, осы өсімдік түрлерінде әртүрлі сипаттағы биологиялық белсенді заттардың болуымен шартталатын, антимутагенді потенциалдың бар екенін көрсетеді.

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

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Изучение антимутагенной активности спиртовых настоев лекарственных растений *Mentha piperíta* L. и *Thymus vulgaris* L. семейства *Lamiaceae*

Глобальное загрязнение среды обитания человека экологически опасными факторами делает актуальным поиск эффективных протекторов природного происхождения для коррекции токсических и генетических эффектов. Одними из перспективных источников таких защитных средств являются комплексы биологически активных веществ, содержащихся в лекарственных растениях. С помощью теста по учету хромосомных аберраций (метафазный метод) на семенах ячменя были изучены мутагенная и антимутагенная активность спиртовых настоев 2-х видов растений семейства *Lamiaceae* мяты перечной (*Mentha piperita* L.) и чабреца обыкновенного (*Thymus vulgaris* L.). В результате проведенных цитогенетических исследований была установлена способность настоев модифицировать уровень индуцированного метилметансульфонатом (ММС) мутагенеза в сторону его снижения. При совместной прямой и обратной обработке семян ячменя растворами спиртовых растительных настоев разной концентрации и классическим мутагеном 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 от концентрации настоя. В работе также были изучены модифицирующие эффекты настоев мяты и чабреца в отношении опасного загрязнителя окружающей среды несимметричного диметилгидразина (НДМГ, 1,1-ДМГ, гептил), основного компонента ракетного топлива, обладающего мутагенной и генотоксической активностью. Как и в экспериментах с ММС, при совместной с НДМГ последовательной обработке тест-объекта спиртовыми настоями мяты и чабреца наблюдалось статистически значимое снижение частоты аберрантных клеток и числа хромосомных аберраций на 100 метафаз ($p < 0,05$), индуцированных несимметричным диметилгидразином. Уровень ингибирования зависел от последовательности воздействия изучаемых агентов и концентрации растительных настоев. Эффективность антимутагенного действия настоев оценивали по редукционному фактору (РФ), который во всех вариантах эксперимента был преимущественно выше 40%. Эти результаты свидетельствует о способности настоев из *Mentha piperita* L. и *Thymus vulgaris* L. ингибировать индуцированный ММС и НДМГ мутагенез более чем на 40%. Проведенные исследования указывают на наличие антимутагенного потенциала у настоев мяты перечной и чабреца обыкновенного, обусловленного наличием биологически активных веществ различной природы в растениях этих видов.

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

Introduction

The global environmental pollution urges the screening of effective approaches to increase the organism's resistance to unfavourable factors including mutagenic and genotoxic agents [1-3]. Long-term complex exposure to various xenobiotics leads to the development of multiple physiological disorders and having an influence upon genetic apparatus leads to potential diseases risk for offspring [4, 5]. Thus, the screening for low-toxic protective compounds is of high actuality. Among them the biologically active compounds (BAC) of medicinal herbs are of interest as they are able to enhance immunity, activate reparative systems, bind free radicals and reactive oxygen species especially [6-13]. Most of crude herbal drugs show wide range of pharmacological activity and relatively low toxicity [14]. In medicinal herbs BAC provide their antioxidant, antimutagenic, immunomodulatory, antiinfllamatory, genoprotective and other effects, thus the medicinal herbs are perspective sources of raw materials for pharmacology [15]. The flora of Kazakhstan is of 6000 species of higher plants and at least 500 species are medicinal herbs [16-18]. Medicinal herbs of *Lamiaceae* family include 250 genera and about 8 thousand species, are of great interest and widely used in folk medicine lavender, basil, mint, thyme, origanum, rosemary, salvia etc. are among them. Such a species as peppermint *Mentha piperíta* L. and common thyme *Thymus vulgaris* L. are most commonly used in folk medicine [19].

Peppermint contains BAC such as menthol, terpenoids (limonene, cineol, dipenten), carotene, rutin, ascorbic acid, ursolic acid, oleanolic acid, flavonoid, tannins, microelements. The folk medicine uses peppermint as a part of herbal collections for the treatment of gastric and bile disorders, nausea, as spasmolytic, antiseptic, astringent, carminative and demulcent remedies and for external use at neuralgia, upper airways illness, burns, hoarseness, bronchitis, heartburn [19].

Species of *Thymus* genera are important essential oil plants and contain phenol compounds as thymol, carvacrol, tannins, bitter stuff, organic pigments, triterpenoids (ursolic, oleanolic acids), terpenes. In medicine, thyme extract is used in treatment of cough, bronchitis, cold, whooping cough, laryngitis and tonsillitis (external use). Essential oil and thymol are part of nonprescription drugs [19].

Screening of medicinal drugs of Trans-Ili Alatau mountain system for antimutagenic and genoprotective activity and the investigation of cellular and molecular aspects of their BAC activity is actual. Advanced researches of herbal tinctures include the identification and recommendation of appropriate species for the creation of antimutagenic herbal collections to reduce the risk of hereditary and oncological diseases. Besides the application of known species could be widened with antimutagenic and genoprotective effects.

The aim of current research was the study of antimutagenic potential of tinctures of medicinal herbs – peppermint *Mentha piperíta* L. and thyme *Thymus vulgaris* L. family *Lamiaceae*. The genetic activity of tinctures was analyzed in two steps: the mutagenic activity of tinctures in different concentrations and protective effects of tinctures under combined mutagen exposure.

Materials and methods

The study was performed on spring two-row barley seeds (*Hordeum vulgare* L.), Baysheshek variety zoned to Almaty region. Barley has low rate of spontaneous mutation and at the same time is susceptible to external exposure thus being a unique test-object for assessment of xenobiotics action. Besides barley contains small number of chromosomes (2n=14) which are of large size (6-8 мкм) [20].

The alcoholic tinctures of peppermint (*Mentha piperíta* L.) in concentrations 1.25; 2.50 and 3.75 ml/L and thyme (*Thymus vulgaris* L.) in concentrations 2.5; 5.0 и 7.5 ml/L were screened for the antimutagenic activity. Aqueous solution of methyl methanesulfonate (MMS) in concentration 5.0 mg/L was used as positive control, and distilled water as negative control. As reference mutagenic xenobiotic unsymmetrical dimethylhidrazine (UDMH) in concentration 5.0 mg/L was used.

The reference mutagen methyl methanesulfonate (MMS, $C_2H_6O_3S$) was used in concentration 5 mg/L [21]. MMS is the strong alkylating agent of direct action that exhibit mutagenic activity in both *in vitro* and *in vivo* tests. In tests on S. *typhimurium* TA 1535/pSK1002 culture it induces SOS-response, in bacteria under absence of metabolic activation induces point mutations. MMS causes somatic and sex-linked lethal mutations in drosophila, induces neoplastic transformation in rodents cell cultures increasing the frequency of sister chromatid

exchanges and chromosomal aberrations. *In vivo* MMS causes mutations in mice germ cells, and *in vitro* in human cells induces formation of micronuclei, DNA single-strand breaks, unscheduled DNA synthesis, gene mutations and sister chromatid exchanges. In rodent somatic cells MMS induces chromosomal aberrations and chromatid exchanges. Due to its broad spectrum of genetic activity methyl methanesulfonate was used as positive control possessing genotoxic and mutagenic activity [21].

Seeds treatment (soaking with agent) was carried out for 4 hours. Seeds were washed out and grown in Petri dishes on water-moistened filter paper under t = 25±1°C in incubator.

Mutagenic and antimutagenic activity of herbal extracts was tested by chromosomal aberration test (metaphase method), preparations and cytogenetic analysis were carried out according to routine methods [22]. 3 hours before fixation grown seeds were transferred into 0,01% colchicine solution to accumulate metaphase plates. Main roots were fixed in ethyl alcohol:glacial acetic acid (1:1) mixture. After the hot hydrolysis with 1N HCl roots were stained with Schiff reactive. Stained roots were washed in three portions of fresh sulphurous water and than subjected to enzymatic maceration by cytase for 40-60 min to separate extracellular matrix and cell walls. Slides were placed on table cooled with liquid nitrogen to -120±1°C for 5-10 min, covering slides were further removed and alcoholic dehydration was performed to obtain stable slide preparations.

The analysis of chromosomal aberrations in cells of root meristeme was performed using microscope Olympus BX 43F (Olympus, Japan). The assessment of structural chromosome abnormalities includes total number of abnormalities and all types of chromosomal aberrations. At least 500 metaphases were scored for each experimental variant. The ability to reduce the frequency of MMS- and UDMH-induced chromosomal aberrations (antimutagenic activity) was assessed by reductional factor (RF). Antimutagenic activity was considered as moderate at inhibition level 25-40%; as strong at more than 40%; as negative at less than 25%.

Statistical analysis was performed using StarPlus and WinPepi softwares. For each variant mean and standard error of mean were calculated. To establish the significance of differences between the mean values of different variants, the Student's test was used. Differences between the data were considered statistically significant at a confidence level of 0.95.

Results

The current study is concerned with antimutagenic effects of alcoholic tinctures of peppermint and common thyme in various concentrations as based on cytogenetic method. As has been mentioned above, the genetic action of tinctures was studied in two steps. First, the mutagenic activity of tinctures in different concentrations was studied in order to select non-mutagenic variants. Further, the DNA-protective properties of selected tinctures were discovered on barley seeds under exposure to mutagen.

The study of mutagenic and antimutagenic activity of alcoholic tinctures of peppermint herb (Mentha piperíta L.) family Lamiaceae. The results of cytogenetic study of primary roots meristematic zone of barley seeds exposed to MMS and peppermint tincture are represented in the Table 1. Rate of spontaneous mutation in seeds grown on distilled water was 1.49±0.52%. The rate of structural mutations in barley seeds induced by MMS significantly exceeded negative control, at that both total frequency of aberrant cells and a number of chromosomal aberrations per 100 metaphases were increased.

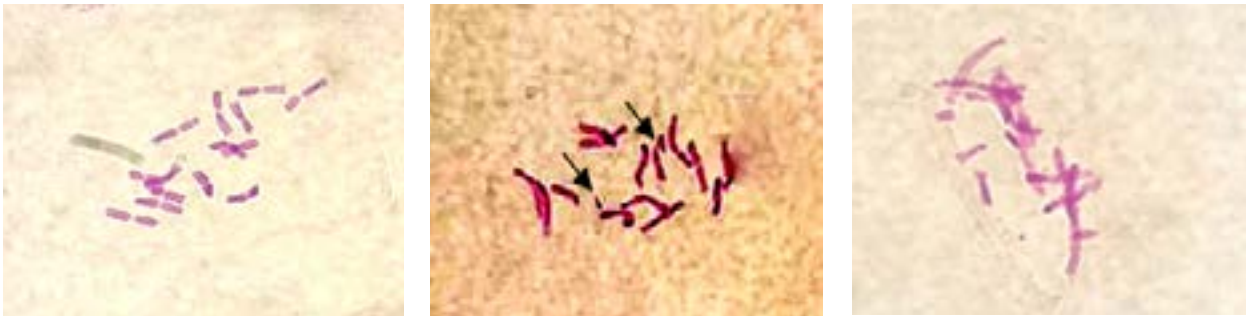
The frequency of aberrant metaphases increased from 1.49% to 5.08% ($p<0.001$) and the number of structural mutations per 100 cells enhanced from 1.49% to 6.03% ($p<0.001$). The rate of chromatid type rearrangements per 100 metaphases significantly increased 7.7 times ($p<0.001$) which is determined by high susceptibility of DNA to MMS action in S-phase and G_2-phase. Among the chromosomal aberrations various rearrangements were observed, but paired and single end-deletions (chromosomal fragments), paired and single interstitial deletions, centric and acentric rings, point fragments prevailed (Figure 1). It should be noted, that a high frequency of anaphases with different types of alterations were observed including chromosomes lag, bridges, single fragments and multipolar mitosis (Figure 2). The frequency of chromosomal rearrangements in the apical meristem of barley seeds exposed to peppermint tinctures in different concentrations did not exceed the negative control level indicating the absence of mutagenic activity of peppermint tinctures.

Further, the ability of peppermint tinctures to modify the mutagenic action of MMS under the combined exposure of barley seeds was studied. Preliminary treatment of seeds with peppermint

tinctures has statistically decreased the yield of MMS induced aberrant cells with increasing solution concentrations in 2.0 ($p<0.05$), 2.5 ($p<0.01$) and 2.4 ($p<0.01$) times and the number of chromosomal aberrations per 100 cells in 2.2 ($p<0.01$), 3.0 ($p<0.01$) and 2.6 ($p<0.01$) times correspondingly.

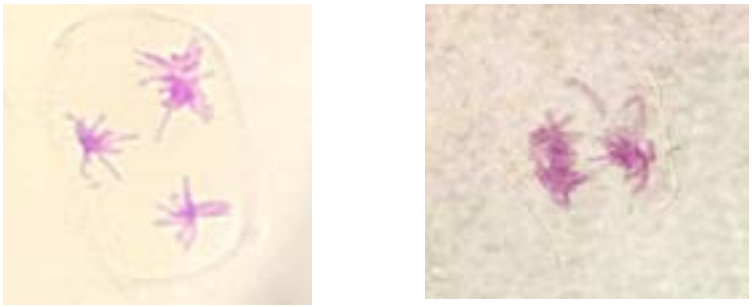
Table 1 – The frequency and range of structural chromosome abnormalities induced in barley seeds under the separate and combined exposure to methyl methanesulfonate and peppermint alcoholic tinctures

Exposure	Total cells studied	Frequency of aberrant cells (M ± m%)	Number of chromosomal aberrations per 100 metaphases		
			total aberrations	chromosomal type	chromatid type
Water	538	1.49 ± 0.52	1.49 ± 0.52	0.93 ± 0.41	0.56 ± 0.32
MMS, 5 mg/L	531	5.08 ± 0.95*	6.03 ± 1.03*	1.69 ± 0.56	4.33 ± 0.88*
Peppermint, 1.25 ml/L	548	1.46 ± 0.51	1.46 ± 0.51	0.73 ± 0.36	0.73 ± 0.36
Peppermint, 2.50 ml/L	524	1.34 ± 0.50	1.34 ± 0.50	0.76 ± 0.38	0.57 ± 0.33
Peppermint, 3.75 ml/L	503	1.59 ± 0.56	1.79 ± 0.59	0.80 ± 0.40	0.99 ± 0.44
Peppermint, 1.25 ml/L + MMS	547	2.56 ± 0.68•	2.74 ± 0.70••	0.55 ± 0.32	2.19 ± 0.63•
Peppermint, 2.50 ml/L + MMS	550	2.00 ± 0.60••	2.00 ± 0.60••	0.36 ± 0.26•	1.64 ± 0.54••
Peppermint, 3.75 ml/L + MMS	566	2.12 ± 0.61••	2.30 ± 0.63••	0.35 ± 0.25•	1.94 ± 0.58••
MMS + peppermint, 1.25 ml/L	512	3.71 ± 0.84	3.91 ± 0.86	0.98 ± 0.43	2.93 ± 0.75
MMS + peppermint, 2.50 ml/L	514	3.11 ± 0.77	3.31 ± 0.79•	0.97 ± 0.43	2.33 ± 0.67
MMS + peppermint, 3.75 ml/L	508	3.54 ± 0.82	3.74 ± 0.84	1.18 ± 0.48	2.56 ± 0.70
Note: * – $p<0.001$ compare to negative control (water); • – $p<0.05$;•• – $p<0.01$ compare to methyl methanesulfonate (MMS)					



Normal chromosome set, 2n =14 Multiple breaks

Figure 1 – Structural chromosome abnormalities induced by MMS, x1000



Multipolar mitosis Chromosome lag

Figure 2 – Anaphases with different types of abnormalities, x1000

Concerning the structural chromosomal aberrations preliminary treatment with peppermint tinctures significantly decreased the yield of MMS induced aberrations of chromatid type ($p<0.01$), and of chromosome type only with second and third tinctures concentrations ($p<0.05$).

The reverse combination when seeds were preliminary soaked in mutagen solution and in peppermint tinctures than, the modifying effect on MMS mutagenic action was different. The frequency of aberrant cells and number of chromosomal aberrations per 100 metaphases as well the rate of chromosomal rearrangements did not differ statistically from MMS exposure only except for followed treatment with tincture in middle concentration where the number of chromosomal aberrations per 100 cells decreased significantly from 6.03 to 3.31 ($p<0.05$). The data obtained testify the ability of alcoholic peppermint tinctures to reduce MMS-induced mutagenesis, at that the decrease in aberrant cells and the number of chromosomal aberrations per 100 cells under preliminary seeds soaking in tinctures was observed.

The efficacy of peppermint antimutageneic action was assessed by reduction factor. Reduction factor of alcoholic tinctura in 1st, 2nd and 3rd concentrations under preliminary seeds soaking was 52.0%, 67.0% and 62.0% correspondingly, testifying more than 50% inhibitory action of *Mentha piperíta* L. tinctura toward MMS induced mutagenesis. Study results give rise to conclusion that assessed peppermint tinctures contain biologically active compounds and have strong antimutagenic action. Only the treatment with tinctura in middle concentration subsequent to MMS exposure significantly decreased the number of chromosomal aberrations per 100 metaphases and reduction factor was 46%.

The study of mutagenic and antimutagenic activity of alcoholic tinctures of Thymus vulgaris L. family Lamiaceae. The results of cytological assessment of mutagenic/antimutagenic activity of thymus tinctures are represented in the Table 2. For all thymus tintcures the genetic activity was not shown. The frequency of aberrant cells and number of chromosomal aberrations per 100 metaphases in roots meristem treated with alcoholic tinctures did not exceed the negative control, meaning the absence of mutagenic activity of thymus extracts in studied concentrations. The ability of thymus tinctures to modify mutagenic activity of MMS was assessed in two ways –tinctures and subsequent mutagen treatment and vice versa.

As can be seen from Table 2 preliminary seeds treatment with thymus tinctures significantly decrease the MMS-induced frequency of aberrant cells and number of chromosomal aberrations per 100 metaphases. MMS induced the frequency of aberrant cells and number of structural mutations up to 5,96% and 6,56% comparing to control. The combined seeds treatment «tinctura + MMS» led to concentration-dependent decrease in 2.0 ($p<0.05$) and 2.1 ($p<0.01$); 2.3 ($p<0.05$) and 2.1 ($p<0.01$); 2.1 ($p<0.05$) and 2.0 ($p<0.01$) times from minimal to maximum concentration correspondingly. It should be noted, that MMS-induced mutagenesis reduction was mainly due to chromosomal aberrations of chromatid type.

Under the reverse seeds treatment «MMS + tinctura» the efficacy of modification of MMS-induced mutagenesis was significantly lower. The only variant where the significant decrease of the number of chromosomal aberrations per 100 metaphases was observed was the treatment with thymus tincture in a middle concentration ($p<0.05$).

Studied alcoholic tinctures of thymus demonstrated the ability to reduce induced mutagenesis, the rate of mutagenesis inhibition depends both on tinctures concentration and treatment sequence. It could be proposed, that biologically active compounds of thymus possess antimutagenic activity. The comparative analysis of results of combined seeds exposure to mutagen and tinctures showed that preliminary treatment with tinctures reduced the rate of induced mutagenesis more effectively.

Reduction factor that was used for the assessment of thyme antimutagenic activity, and in case of preliminary tinctures treatment in all studied concentrations RF was more than 50% indicating the ability of thyme tinctures to inhibit MMS-induced mutagenesis. Reduction factor was assessed only in those variants where the statistically significant decrease of induced mutagenesis had been shown, thus at MMS exposure followed by treatment with middle concentration extracts RF was 43%.

The modification of UDMH-induced mutagenesis by alcoholic tinctures of medicinal herbs Mentha piperíta L. and Thymus vulgaris L.

Table 2 – The frequency and range of structural chromosome abnormalities induced in barley seeds under the separate and combined exposure to methyl methanesulfonate and thymus alcoholic tinctures

Exposure	Total cells studied	Frequency of aberrant cells (M ± m%)	Number of chromosomal aberrations per 100 metaphases		
			total aberrations	chromosomal type	chromatid type
Water	550	1.45 ± 0.51	1.49 ± 0.52	0.93 ± 0.41	0.56 ± 0.32
MMS	503	5.96 ± 1.06*	6.56 ± 1.10*	1.99 ± 0.62	4.57 ± 0.93*
Thymus, 2.5 ml/L	504	1.39 ± 0.52	1.39 ± 0.52	0.79 ± 0.40	0.60 ± 0.34
Thymus, 5.0 ml/L	510	1.57 ± 0.55	1.57 ± 0.55	0.98 ± 0.44	0.59 ± 0.34
Thymus, 7.5 ml/L	530	1.51 ± 0.53	1.51 ± 0.53	0.75 ± 0.38	0.75 ± 0.38
Thymus, 2.5 ml/L + MMS	515	2.91 ± 0.74•	3.11 ± 0.76••	1.17 ± 0.47	1.94 ± 0.61•
Thymus, 5.0 ml/L + MMS	518	2.90 ± 0.74•	3.09 ± 0.76••	1.54 ± 0.54	1.54 ± 0.54••
Thymus, 7.5 ml/L + MMS	520	2.88 ± 0.73•	3.27 ± 0.78•	1.35 ± 0.51	1.92 ± 0.60•
MMS + Thymus, 2.5 ml/L	515	3.88 ± 0.85	4.08 ± 0.87	1.55 ± 0.54	2.52 ± 0.69
MMS + Thymus, 5.0 ml/L	508	3.54 ± 0.82	3.74 ± 0.84•	1.57 ± 0.55	2.17 ± 0.65•
MMS + Thymus, 7.5 ml/L	520	3.46 ± 0.80	3.46 ± 0.80	1.35 ± 0.51	2.12 ± 0.63
Note: * – $p<0.001$ compare to negative control (water); • – $p<0.05$;•• – $p<0.01$ compare to methyl methanesulfonate (MMS)					

The component of rocket fuel unsymmetrical dimethylhydrazine (UDMH, 1,1-dimethylhydrazine) is the hazardous pollutant contaminating the atmosphere, soils and subsoil waters during the emergency booster launching, at Baikonur Cosmodrome in particular. There are multiple researches indicating the toxic, genotoxic and mutagenic activity of UDMH in both plant and animal cells [23, 24].

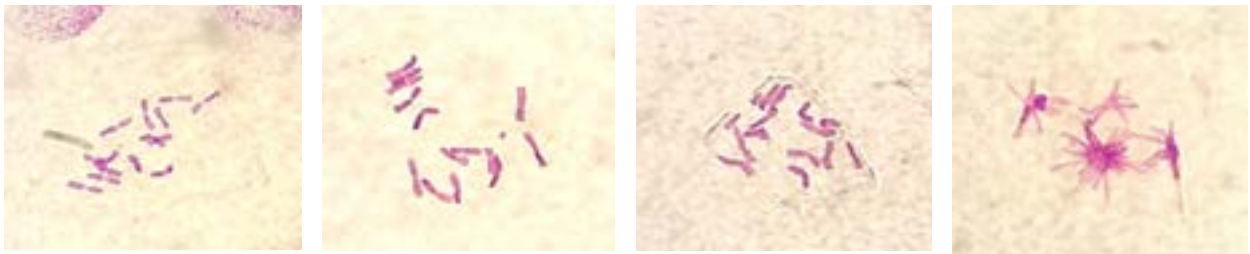
In current research, the tinctures of peppermint and thyme that showed antimutagenic activity were appraised under UDMH-induced mutagenesis. As shown in Table 3 UDMH significantly increased the frequency of aberrant cells ($p<0.01$) and the number of chromosomal aberrations per 100 metaphases ($p<0.001$) when compared to negative control. Among the observed chromosomal abnormalities, the chromatid aberrations prevailed, however increased frequency of chromosomal rearrangements and anaphase alterations were observed (Figure 3).

The combined seeds treatment «peppermint+UDMH» statistically significant decreased UDMH-induced mutagenesis. Preliminary seeds soaking in peppermint extracts in all concentrations led to decrease in total frequency of aberrant cells and number of chromosomal aberrations per 100 metaphase in 2.1 and 2.2 times (low concentration, $p<0.05$); in 2.3 and 2.4 times (middle concentration, $p<0,01$); in 2.2 and 2.3 times (high concentration, $p<0.01$). At that significantly decreased the frequency of UDMH-induced structural mutations of chromatid type. The same picture was observed under preliminary UDMH exposure «UDMH+peppermint», however the statistically significant decrease of UDMH-induced mutagenesis was observed only under treatment with tinctures in middle and high concentrations ($p<0.05$).

Under the combined seeds exposure «thyme+UDMH» there was observed the statistically significant reduce of UDMH-induced mutagenesis. Preliminary treatment of seeds with thyme extract in all studied concentrations led to decrease in total frequency of aberrant cells and number of chromosomal aberrations per 100 metaphases in 2.5 and 2.1 times (low concentration, $p<0.05$); in 2.4 and 2.4 times (middle concentration; $p<0.05$; $p<0.01$); in 2.3 and 2.2 times (high concentration, $p<0.05$). As in experiments with peppermint, statistically significant decrease in the frequency of chromatid type mutations induced by unsymmetrical dimethylhydrazine was observed. Thyme extract treatment followed the exposure to UDMH (UDMH+thyme) decreased the UDMH-induced frequency of aberrant cells in all three concentrations ($p<0.05$).

Table 3 – The frequency and range of structural chromosome abnormalities induced in barley seeds under the separate and combined exposure to unsymmetrical dimethylhydrazine and peppermint and thymes alcoholic tinctures

Exposure	Total cells studied	Frequency of aberrant cells (M ± m%)	Number of chromosomal aberrations per 100 metaphases		
			total aberrations	chromosomal type	chromatid type
Water	538	1.49 ± 0.52	1.49 ± 0.52	0.93 ± 0.41	0.56 ± 0.32
UDMH	509	4.91 ± 0.96*	5.50 ± 1.01**	1.97 ± 0.62	3.54 ± 0.82**
Peppermint, 1.25 ml/L + UDMH	518	2.32 ± 0.66•	2.51 ± 0.69••	1.16 ± 0.47	1.35 ± 0.51•
Peppermint, 2.50 ml/L + UDMH	515	2.14 ± 0.64•	2.33 ± 0.66••	0.97 ± 0.43	1.36 ± 0.51•
Peppermint, 3.75 ml/L + UDMH	530	2.26 ± 0.65•	2.45 ± 0.67•	1.13 ± 0.46	1.32 ± 0.50•
UDMH + Peppermint, 1.25 ml/L	514	3.11 ± 0.77	3.50 ± 0.81	1.36 ± 0.51	2.14 ± 0.64
UDMH + Peppermint, 2.50 ml/L	525	2.48 ± 0.68•	2.86 ± 0.73•	1.14 ± 0.46	1.71 ± 0.57
UDMH + Peppermint, 3.75 ml/L	545	2.57 ± 0.68•	2.75 ± 0.70•	1.10 ± 0.45	1.65 ± 0.55
Thymus, 2.5 ml/L + UDMH	500	2.00 ± 0.63•	2.60 ± 0.71•	1.20 ± 0.49	1.40 ± 0.53•
Thymus, 5.0 ml/L + UDMH	530	2.08 ± 0.62•	2.26 ± 0.65••	0.75 ± 0.38	1.51 ± 0.53•
Thymus, 7.5 ml/L + UDMH	515	2.14 ± 0.64•	2.52 ± 0.69•	0.97 ± 0.43	1.55 ± 0.54•
UDMH + Thymus, 2.5 ml/L	548	2.55 ± 0.67•	2.74 ± 0.70•	1.09 ± 0.44	1.82 ± 0.57
UDMH + Thymus, 5.0 ml/L	552	2.36 ± 0.65•	2.90 ± 0.71	1.09 ± 0.44	1.81 ± 0.57
UDMH + Thymus, 7.5 ml/L	528	2.46 ± 0.67•	2.84 ± 0.72•	0.95 ± 0.42	1.89 ± 0.59
Note: * – p<0,001 compare to negative control (water); • – p<0,05;•• – p<0,01 compare to UDMH					



Normal chromosome set, 2n =14 | Microfragment | Paired end deletion | Multipolar mitosis

Figure 3 – Structural chromosome abnormalities induced by UDMH, x1000

Reduction factor of peppermint and thyme alcoholic tinctures in all concentrations both under preliminary or followed UDMH exposure exceeded 40%. Data obtained indicated the antimutagenic potential *Mentha piperita* and *Thymus vulgaris* and necessity of further researches on laboratory animals.

Discussion

Widespread industrialization, use of chemical technologies and a variety of artificially synthesized chemicals in various spheres of human economic activity increase the genetic risk for the population. However, it is almost impossible to completely avoid human contact with mutagenic factors in everyday life. Therefore, one of the priority tasks is to ensure genetic safety for the population. In this regard, of particular interest is the issue of antimutagenesis, which is defined as a biological phenomenon of suppression of both spontaneous and induced mutagenesis [25].

One of the promising approaches to reduce the negative effect of environmental mutagens on the human body is the use of various biologically active substances with antimutagenic potential. This approach seems to be a possible and practically feasible way to prevent genotoxic effects; therefore,

antimutagens can be used in the genetic safety system as genome protectors [25].

In current work the cytogenetic researches on plant test-object, barley (*Hordeum vulgare* L.) were carried out to investigate the mutagenic and antimutagenic activity of tinctures of two medicinal herbs of family *Lamiaceae* – peppermint (*Mentha piperíta* L.) and thyme (*Thymus vulgaris* L.). Studied plant extracts showed no mutagenic activity in barley seeds and demonstrated the antimutagenic activity under the mutagen exposure. Antimutagenic activity was revealed as statistically significant inhibition of MMS- and UDMH-induced mutagenesis. The rate of induced mutagenesis as decrease in frequency of chromosomal aberrations depended both on treatment sequence and tinctures concentration.

At present, a fairly large number of antimutagens of various nature have been discovered. The mechanism of action of many of them is still not fully understood, due to which they have not found widespread use. In addition, their harmlessness to the human organism has not been fully assessed, and traditional pharmacotoxicological studies of antimutagens have not been carried out.

In this regard, the search for inhibitors of induced mutagenesis among approved drugs and medicinal plants widely used in folk medicine is promising. Their pharmacological activity is due to a wide range of biologically active substances contained in plant raw materials, most important of these are alkaloids, cardiac glycosides, saponins, tannins, resins, essential oils, gums, vitamins, phytoncides, etc [26].

Among the biologically active substances in medicinal plants, phenolic compounds are distinguished into a separate class: phenylpropanoids, which include several structurally different groups: glycosylated phenylpropanoids, flavonoids, isoflavonoids, coumarins, stilbenoids, curcuminoids and lignans [27]. Numerous studies of the biological and pharmacological properties of phenylpropanoids indicate that they are promising for use as components of immunomodulatory and antioxidant drugs [28-31].

The genetic effects of most chemical mutagens are manifested through the development of oxidative stress, therefore, most of the known antimutagens are characterized by antioxidant activity [32]. The studied extracts of peppermint and common thyme contain phenolic and polyphenolic compounds that can inhibit free radical processes. Some phenols are capable of inhibiting the formation of mutagens from their precursors. Tannins are found in many perennial plants and contain more phenolic OH groups, which allows them to firmly bind to proteins and other biopolymers. In addition, they are able to bind toxins and heavy metal salts. These properties of tannins can significantly reduce the induced mutability [33].

As for the mechanisms of action of antimutagens, they can be different. Most antimutagens are biologically active substances that can act on nucleic acids. It is safe to say that the antimutagenic activity of most biologically active substances of plant origin is primarily due to the ability to suppress free radical processes induced by the action of genotoxicants and to activate the repair systems.

The further researches on antimutagenic and gene-protective action of peppermint and thyme tinctures are planned using the animal test-objects.

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