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INTRASPECIFIC SPONTANEOUS CHROMOSOMAL MUTATIONS IN SOME RODENTS DISTRIBUTED IN AZERBAIJAN, THEIR INDUCTION BY IONIZING RADIATION AND CORRECTION

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Aim. *Work is focused on the intraspecific spontaneous mutations of rodent chromosomes common in different regions of Azerbaijan, the effect of gamma radiation in doses 1 and 4 Gy on chromosomal aberrations in the bone marrow of rats pretreated with an extract from the collection of medicinal plants by injection was studied.* ***Methods.*** *The works on the study of chromosome morphology, changes in heterochromatic parts of chromosomes, chromosome regions of nucleoli formation, biometric processing of chromosomal aberrations.* ***Results.*** *Intrapopulational spontaneous chromosomal mutations in ordinary field mice in the Lesser Caucasus were registered. In normal karyotype chromosomes of fifth pair are presented by large subtelocentrics, while one chromosome of fifth pair in abnormal karyotype was heteromorphic and had acrocentric structure. Heteromorphic chromosomes differ in the heterochromatin composition as well. In the process of speciation are found mainly pericentric inversions, Robertson and tandem translocations. In metaphase plates along with chromosomal mutations were detected structural heterochromatin and nucleoli formation regions. Extract from the collection of medicinal plants was found to promote reduction of structural chromosome abnormalities as a result of radiation and ensures the normal functioning of cells.* ***Conclusions.*** *Spontaneous mutations in the fifth pair of chromosomes could be considered as the beginning of speciation, but recent studies in the Lesser Caucasus did not found individuals with this type of mutation. Microtus mayori karyotype demonstrated associations in nucleoli formation regions and it was suggested their mechanism.*

Key words: *chromosome, wild mouse, extract, karyotype, radiation.*

Introduction. Since the middle of XX century after the first karyological investigations it was revealed that, the main role in the formation of species and differences among the populations belongs to chromosomal and genomic mutations. Mutation process along with its spontaneous character also depends on a number of factors. It is known that in addition to other adverse environmental factors

damages of different character in living organisms due to irradiation impact occur. Depending on the radiation dose normal running of biochemical processes are disturbed, intermediate toxic substances are formed, synthesis of proteins gets down-regulated, breaks in somatic and sex chromosomes happen, and as a result different defects in further generations due to the remote influence of radiation are formed. The carrier of genetic information in the cellular level are chromosomes, so structural changes in chromosomes during the formation of sex cells and radiation damage gives start to different pathologies and hereditary disorders. Formation of different type of chromosomal aberrations depends on strength of irradiation impact.

To minimizing impact of different kinds of radiation, producing and investigation of effects of novel biopreparations is recently considered to be one of the actual problems. For this purposes preference is given to studies of substances of plant origin that increase radioprotection, accelerate the metabolic processes in an organism, possess efficiency in pro-longed use and devoid of toxic effects.

With growth of number of investigations the types of chromosomal changes were identified. Elaboration and application of methods of differential staining has showed that formation of species, differences among the populations and inside the populations form not only as the result of pericentric inversions and genomic mutations. Along with chromosomal mutations like pericentric inversions, Robertson translocations, structure of constutive heterochromatine that is observed with tandem translocations, centromere, intercalary and whole arms, NFR (nucleolus-forming regions) are as well of great importance [1 – 3]. Chromosomal associations – connection of two chromosomes via centromeres and centromere-telomere parts [4] could be accepted as a change that occurs inside the population. Though the shown mutations have a normal character, on the other hand they cause abnormal changes.

The aim of the investigation is intra-species spontaneous chromosomal aberrations in some rodents spread in Azerbaijan and their formation as a result of radiation impact.

Part of the material were collected from the Great Caucasus (*Microtus mayori*) and the Little Caucasus (*Microtus obscurus*) areas.

Materials for Microtus obscurus were taken around the Gedebey city in the Little Caucasus *Microtus obscurus* – ordinary field mouse 13 samples (8♀♀; 5♂♂), *Microtus mayori* – Caucasian shrub field mouse or Little-Asian shrub field mouse 22 samples (9♀♀; 13♂♂), and 6 samples (3♀♀; 3♂♂) were collected from Gebzdere field of Zagatala region, the Great Caucasus.

Materials and Methods

The studies with γ-irradiation were carried out on three-month-old Wistar rats of 100–120 g weight that reached sexual maturity. There were used 16 animals in experiments (8 ♀, 8 ♂). Before irradiation exposure the extract rich with biological active substances that were got from the plant composition were injected to animals inraperitoneally in a dose of 70 mg/kg. After 2 h animals were subjected to γ-irradiation in the apparatus “Rkhund”, at a dose rate 0.3 rad/sec, at 1 and 4 Gray. After a day from irradiation chromosomal disturbances in the bone marrow cells were observed. For analysis of chromosome set XSZ–158 microscope for biological studies with software was used.

In the experiments ordinary staining [5], G-staining [6], C-staining [7], nucleolus-forming regions staining [8] methods were used.

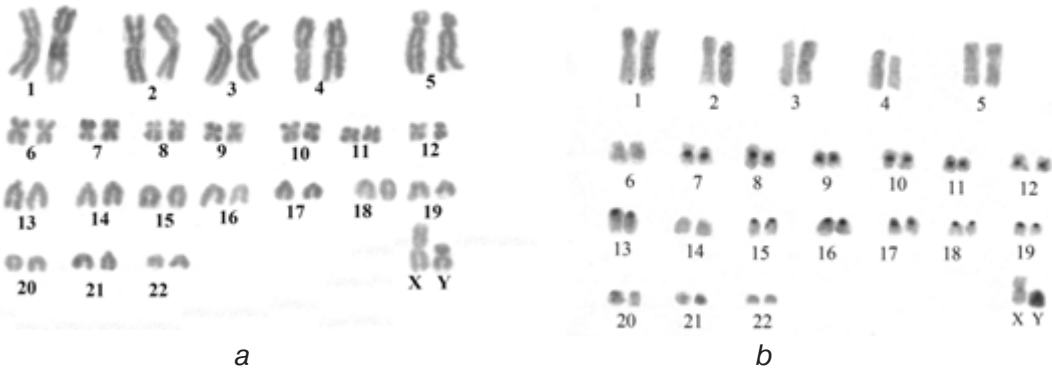
Results and Discussion

Normal karyotype of ordinary field mouse. There were diploid chromosome number 2n=46 in all investigated samples. Excluding anomalous individuals in all individuals total number of main arms were NF=72. There were observed 5 pairs of big and 17 pairs of little autosome chromosomes in chromosomal complement of normal karyotype. Big chromosome group consists of 2 pairs of metacentric, 2 submetacentric and one subtelocentric chromosomes. In the little chromosome group 7 pairs of double- arms (meta- and submetacentric) and 10 pairs of chromosomes of acrocentric structure are identified. Y-chromosome is one of the acrocentric chromosomes by size (Figure 1*a*).

By the means of G-staining each chromosome is clearly identified. The band structures of big chromosomes differ very clearly from each other. There are defined from one up to three bands in the most of the little chromosomes. In every arm of X-chromosome there is one band near the centromere part. There is observed one clear band around the centromere part of Y-chromosome.

Heterochromatin is not defined in all chromosomes in karyotype of ordinary field mouse. This compound can be found mainly in the centromere part in chromosomes that contains heterochromatin. In big chromosomes and X chromosome the C-stain is not found. Heterochromatin could be found in the centromere part of only in 6 pair of double-arms and 6 pair of acrocentric chromosomes of the 17 pair of little chromosomes. Y-chromosome possesses completely heterochromatin structure [9].

There was defined abnormal karyotype in five individuals of ordinary field mice that were caught from around of Gedebey city. One of them was male. The 5th pair of the chromosome of these individuals had a heteromorphic structure (in normal karyotype each one of 5th pair of chromosome has subtelocentric form). One of the homologs of 5th pair of abnormal chromosome has subteloecentric, other one has acrocentric structure (Figure 2*a*). On the basis of G-band contours of these two chromosomes show that a pericentric inversion could be accepted as a reason of passage of subtelocentric structure into acrocentric one in one of the chromosomes. The region around the centromere of inversion chromosome is not exposed to G-staining and as a result is not stained. In case of C-stain this region is stained intensively and also it was determined that there is a big heterochromatin block in the acrocentric chromosome that is formed as a result of inversion (Figure 2*b*). It should be noted that hetero-



a *b*

Figure 1. Karyotype of ordinary field mouse. *a* – ordinary stain, *b* – C-stain.

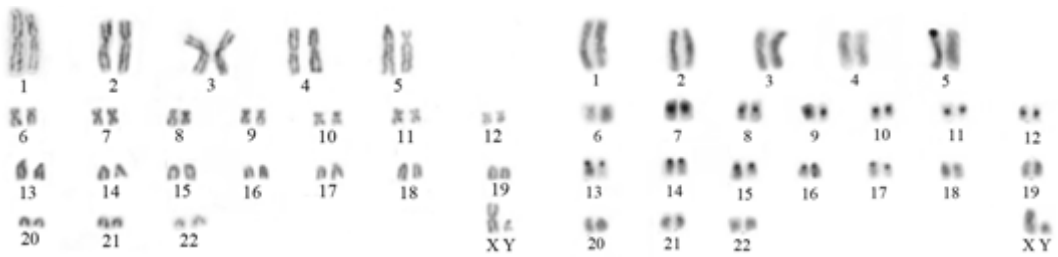
chromatin blocks are defined in the centromere zones of 6 pairs of metacentric and 5 pairs of acrocentric chromosomes of autosome chromosomes in individuals with abnormal karyotype. By this index they differ from individuals with normal karyotype (as told before there is defined heterochromatin blocks in the centromere of 12 pair of chromosomes in normal karyotype). Therefore it is possible that heterochromatin blocks of little autosome chromosomes take part in formation of big heterochromatin centromere block of newly formed acrocentric chromosome of the 5th pair of chromosome.

The heteromorphism that is determined in the 5th pair of chromosomes in karyotype of *Microtus obscurus* ordinary field mouse beside the Gedebey region, is also found in Khosrov reserve, Spitak, Kokchtav region of Kazakhstan, Novosibirsk (Academic campus), Ust-bakchari region of Tomsk province. It is registered in 4 individuals of 13 ones and in Azerbaijan in 5 individuals of 103 ones in Gedebey polulation [9].

The heteromorphism that has been defined by us in the 5th pair of chromosomes constitutes approximately 4,87 percent of overall number of investigated individuals (in the Little Caucasus 31,25 %). In different populations that were investigated last years, also in animals that were caught from Gedebey region heteromorphism in the 5th pair of chromosome were not registered. It is assumed that influence of factors of anthropogenic origin and change of ecological situation, also food ration and the influence of other unknown factors in these years realized adverse effects on heteromorph animals.

Microtus (Terricola) majori – Caucasian shrub field mouse. The karyotype of Caucasian shrub field mice were investigated in two populations of Gedebey region and in Zagatala region. The diploid chromosome number in all individuals of three populations are 2n=54. The number of main arms are NF=60. The first and second pairs of chromosomes are double-armed in karyotype. The rest 24 pairs of chromosomes are in acrocentric structure and form a reducing line by size. In karyotype X-chromosome is a big submetacentric. Y-chromosome is middle-sized acrocentric.

By the means of differential staining method the G-band is clearly identified in autosome and sex chromosomes. Approximately in the middle part of upper arm of X-chromosome an intensive stained wide band is determined. The centromere part of chromosome stains very intensive and is in a dark color. Three weak stained bands appear in long arm of X-chromosome, although one of them is close to centromere but is localized distantly from the centromere. The second band is situated in the side of telomere part from the middle part of long arm and relatively to other two bands it is stained weakly and at least is double wide. The second band is narrow and is lo-



a *b*

Figure 2. Karyotype of ordinary field mouse. *a* – ordinary stain, *b* – C- stain.

calized in the telomere part of long arm. Two bands in Y-chromosome are defined. Both of them are nearly of a same width. One of them is situated near the centromere part, other one is near the telomere part.

In individuals that were caught from Zagatala region the nucleolus-forming regions were defined in 6th and 8th pairs of big chromosomes, in 9th, 10th 13th pairs of middle sized chromosomes, and in 20th pair of little chromosomes. In 18th and 24th pairs of chromosomes nucleolus-forming regions are defined weakly. In the rest autosome chromosomes, and in sex chromosomes nucleolus forming regions are not defined. There are identified chromosomal associations between themselves of 6th and 9th pair of homological chromosomes (Figure 3).

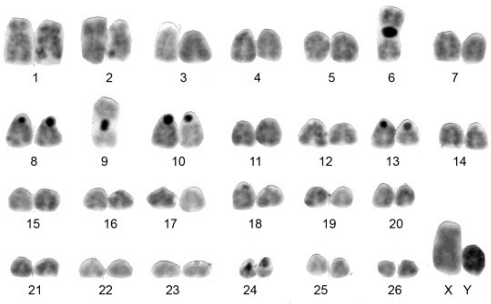


Figure 3. Karyotype of Microtus mayori. White stain.

Chromosomal associations could occur both between homologous and non-homologous chromosomes. In Figure 3, association between themselves of 6th pair of chromosomes and the same between 9th pair of chromosomes is registered. We know very few sources of information about chromosome associations. Although there is information about chromosome association in literature, there is not any information about its mechanism, its reasons. We suppose that, chromosomal associations could be one of the factors causing intrapopulation polymorphism [10].

Usually number of spontaneous mutations in mammalian populations are little. Therefore information about types of spontaneous chromosomal changes is rarely met. However under the influence of radiation structural chromosomal changes form rapidly and therefore information can be quickly collected.

We think that, that in addition to spontaneous mutations, comparative investigation of influence of irradiation of different doses, which is one of the environmental factors, and correction of formed damages with the means of plant extracts will contribute to researches carrying out in the direction of resolving of problem of radiation safety. In our earlier investigations, radioprotector effect of extracts obtained from some medicinal plants of Azerbaijan were studied and the results were printed in scientific journals [11, 12].

This time, the purpose of the investigation is to study the influence of extract obtained from collection of calendula (*Calendula officinalis* L.), swallowwort (*Chelidonium majus* L.) and sage (*Salvia officinalis*) (CSS) on chromosomal aberrations.

We carried out extraction from an alcoholic solution in boiling at reflux ball-bearing condenser. Evaporation of solvent was carried out in vacuum in a rotary evaporator at 30–40˚ C until obtaining of pappy residue.

During the cytogenetic analysis of bone marrow and germ cells of control Wistar rats chromosomal disorders were not detected, also injection of the extract was not seriously affected number of spontaneous mutations. For each individual there were investigated not less than 200 bone marrow cell metaphase plates.

The obtained results are statistically significant [13].

By the increase of radiation dose division of cells is weakened. At 1 Gy dose of γ-irradiation number of chromosomal aberra-

Table 1. Characteristics of investigation material and types of influence

Types of influence	Number of rats	Number of observed mitotic cells
Intact control	1 ♂+ 1 ♀	520
1 Gray γ-irradiation	1 ♂+ 1 ♀	550
4 Gray γ-irradiation	1 ♂+ 1 ♀	490
Extract of plant collection (CSS)	1 ♂+ 1 ♀	548
Extract (CSS) + 1 Gy	2 ♂+ 2 ♀	527
Extract (CSS) + 4 Gy	2 ♂+ 2 ♀	555

Table 2. Influence of 1 and 4 Gy γ-irradiation and plant collection extract (CSS) on incidence of chromosomal aberrations in bone marrow cells in rats

Variants	Number of metaphases (total)	Number of metaphases 2n=42	Poliploid metaphases	Aneuploid metaphases	Structural disorders	Disorders %
Intact control	520	516	2	1 (n>42)	1	0,76±0,08 P±<0,02
1 Gray γ-irradiation	550	533	4	4(n>42) 3(n<42)	6	3,09±0,77 P<0,05
4 Gray γ-irradiation	490	447	13	7(n>42) 4(n<42)	19	8,77±1,08 P<0,04
Extract of plant collection (CSS)	548	538	3	5(n<42)	2	1,82±1,05 P<0,01
Extract (CSS) + 1 Gy	527	516	1	4(n>42) 2(n<42)	4	2,08±0,91 P<0,05
Extract (CSS) + 4 Gy	555	517	9	6(n>42) 7(n<42)	16	6,84±1,09 P<0,03

tions increases by over 3,09 %. Irradiation at a dose 4 Gy increases the number of aberrations up to 8,77 %. This growth is mostly due to the structural damages. After the injection of collection extract the number of chromosomal aberrations decrease a bit and reaches 2,08 % in 1 Gy-irradiated rats. Also in rats subjected to irradiation at a dose 4 Gy the number of aberrations decreases from 8,77 % to 6,84 %. Under the influence of collection extract weakening of cell division was not observed.

It has been shown, that plant collection extract decreases the mutagenic effect of γ-irradiation, restores rate of cell division, reduces the number of chromosomal aberrations. This indicates to the possibility of use of the collection extract in corrections of radiation damages.

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ВНУТРИВИДОВЫЕ СПОНТАННЫЕ ХРОМОСОМНЫЕ МУТАЦИИ У НЕКОТОРЫХ ГРЫЗУНОВ, РАСПРОСТРАНЕННЫХ В АЗЕРБАЙДЖАНЕ, ИНДУКЦИЯ ИХ ИОНИЗИРУЮЩИМ ИЗЛУЧЕНИЕМ И КОРРЕКЦИЯ

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Цель. В работе исследованы внутривидовые спонтанные мутации хромосом грызунов, распространенных в различных районах Азербайджана, изучено влияние гамма излучения в дозах 1 и 4 грей на хромосомные аберрации в костном мозге крыс, предварительно получивших экстракт из сбора лекарственных растений путем инъекции. **Методы.** Выполнены работы по изучению морфологии хромосом, изменений гетерохроматиновых частей хромосом, районов образования ядрышек хромосом, биометрическая обработка хромосомных аберраций. **Результаты.** Отмечены внутрипопуляционные спонтанные хромосомные мутации у обыкновенных полевых мышей на Малом Кавказе. В нормальном кариотипе хромосомы пятой пары большие субтелоцентрические, в то время как в анормальном кариотипе одна из пятой пары хромосом была гетероморфной и имела акроцентрическое строение. Гетерохромные хромосомы отличаются и по гетерохроматинному составу. В процессе видообразования отмечаются в основном перицентрические инверции, транслокации Робертсона и тандемные транслокации. В метафазных дисках наряду с хромосомными мутациями обнаружены структурный гетерохроматин и районы образования ядрышек. Выявлено, что экстракт из сбора лекарственных растений способствует снижению структурных нарушений хромосом образуемых под действием радиации и обеспечивает нормальное функционирование клетки. **Выводы.** Спонтанные мутации в пятой паре хромосом можно было рассмотреть как начало видообразования, однако в последних исследованиях на Малом Кавказе не обнаружены особи, имеющие такой тип мутации. В кариотипе вида *Microtus mayori* обнаружены ассоциации в районах образования ядрышек, отмечены межхромосомные ассоциации и

высказано предположение о механизме ассоциации.

Ключевые слова: хромосома, дикая мышь, экстракт, кариотип, радиация.

ВНУТРІШНЬОВИДОВІ СПОНТАННІ ХРОМОСОМНІ МУТАЦІЇ У ДЕЯКИХ ГРИЗУНІВ, ПОШИРЕНИХ В АЗЕРБАЙДЖАНІ, ІНДУКЦІЯ ЇХ ІОНІЗУЮЧИМ ВИПРОМІНЮВАННЯМ І КОРЕКЦІЯ

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Мета. У роботі досліджено внутрішньовидові спонтанні мутації хромосом гризунів, поширених у різних районах Азербайджану, вивчено вплив гамма випромінювання в дозах 1 і 4 грей на хромосомні аберації в кістковому мозку щурів, які попередньо отримали екстракт зі збору лікарських рослин шляхом ін'єкції. **Методи.** Виконано роботи з вивчення морфології хромосом, змін гетерохроматинових частин хромосом, районів утворення ядерець хромосом, біометрична обробка хромосомних аберацій. **Результати.** Відзначено внутрішньопопуляційні спонтанні хромосомні мутації у звичайних польових мишей на Малому Кавказі. У нормальному каріотипі хромосоми п'ятої пари великі субтелоцентрічні, тоді як в анормальному кариотипі одна з п'ятої пари хромосом була гетероморфною і мала акроцентричну будову. Гетерохромні хромосоми відрізняються і за гетерохромативним складом. У процесі видоутворення відзначаються в основному перицентричні інверсії, транслокації Робертсона і тандемні транслокації. У метафазних дисках поряд з хромосомними мутаціями виявлені структурний гетерохроматин і райони утворення ядерець. Виявлено, що екстракт із збору лікарських рослин сприяє зниженню структурних порушень хромосом утворених під дією радіації і забезпечує нормальне функціонування клітини. **Висновки.** Спонтанні мутації в п'ятій парі хромосом можна було розглянути як початок видоутворення, проте в останніх дослідженнях на Малому Кавказі не виявлені особини, що мають такий тип мутації. У каріотипі виду *Microtus mayori* виявлені асоціації в районах утворення ядерець, відзначені міжхромосомні асоціації та висловлено припущення про механізм асоціації.

Ключові слова: хромосома, дика миша, екстракт, каріотип, радіація.