Section 5

Molecular genetics

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ESTIMATION AND ASSESSMENT OF CYTOGENETIC CHANGES IN BONE MARROW CELLS OF LABORATORY ANIMALS RECEIVED A GENE-MODIFIED PRODUCT

Current trends in the change in the structure of morbidity indicate an increase in the relative importance of

genetically determined diseases. The results of biological monitoring of human populations have shown that at present there is not only an increase in hereditary pathology, but also an increase in the frequency of diseases with a genetic component. The aim was to study cytogenetic changes in the cells of the bone marrow of laboratory animals that received and did not receive GMO soy. It is found in white mongrel rats treated with GMO soybean detected

cytogenetic changes in their red om bone ohm brain e lead partially m the inhibition and proliferation of bone marrow cells. Of all be analyzed metaphases among them revealed altered karyotypes – polyploidy (5.6%) and aneuploidy (5.6%). In the remaining groups (group 2 and 3), no such changes were observed. In the group of animals that did not receive soy with and without GMO, all bone mar- row samples had only metaphase plates with a normal karyotype, and the cells did not contain genomic abnormalities.

Key words: genetically modified soybeans, cytogenetic changes, bone marrow, polyploidy, aneuploidy, experiment.

Introduction

Genetically modified (GM) organisms are plant or animal organisms, the genotype of which has been changed in a way that is not natural for nature, using methods of genetic engineering to give the body new properties: resistance to herbicides, pests, diseases and salinity, to the action of high and low temperatures, increase calories; to solve the problems of cleaning the environment from organic pollution and heavy metals; to ensure the synthesis of certain compounds in the plant organism and the use of plants for the production of these compounds [1, 2, 3, 4].

Genetic changes are made for scientific or economic purposesand distinguished by a purposeful change in the genotype of an organism, in contrast to the random, characteristic of a natural mutation process. The main trait inherent in most GMO plants is herbicide and pest resistance. Cultivation of GM-soybeans does not have a single positive moment [5, 6, 7].

The most serious risks associated with products of genetic engineering are combined into 2 main groups: food and Ecological [8, 9].

Food – weakening of the immune system; the occurrence of allergic reactions; the development of pathologies associated with the accumulation of pesticides in the human body, introduced by the used GMO products,

Ecological – the loss of the diversity of thegene pool of wild relatives of cultivated plants due to their cross-pollination with related GM plants; contamination of water resources by the use of pesticides; depletion and disturbance of natural soil fertility associated with the suppression of GM plants by toxins, the vital activity of soil invertebrates and microflora; acceleration of the second plant

growth to a much greater extent than in traditional agricultural RMS households Affiliations cultures. In general, the above studies indicate the insufficient substantiation of the safety of GMO plant products for humans and the environment.

Current trends in the change in the structure of morbidity indicate an increase in the relative importance of genetically determined diseases [9, 10].

Many authors believe that now important is to solve the urgent problems and correlating the rate of changes in the environment with the ability to adapt human populations, in addition it is necessary to assess the extent to which, in the biosphere changes that affect the rate of mutation [6, 11, 12].

The results of biological monitoring of human populations have shown that at present there is not only an increase in hereditary pathology, but also an increase in the frequency of diseases with a genetic component. The danger of induced mutagenesis is that newly emerging mutations have a negative impact on the fitness of the population and on the health of the population.

In this regard, new research in this direction is relevant and in demand at the present time.

The aim of the study was to learn the evaluation cytogenetic changes in bone marrow cells in laboratory animals, treated and not treated with GMO-product in a comparative aspect.

Materials and Methods

To perform the planned research used 30 white outbred rats weighing bodies 150-180 g of both sexes, contained in a standardized vivarium conditions-with relative humidity 50-60%, temperature vivarium 22 0 C, light conditions (12 h aces darkness and light). The maintenance of laboratory animals, feeding and caring for them, selection of animals, cleaning and disinfection of the vivarium premises were carried out in accordance with the approved recommendations [13]. All laboratory animals were obtained from the same nursery and were of the same age. All laboratory animals were quarantined for 21 days prior to the start of experimental studies. When working with experimental animals, all ethical principles of working with laboratoryanimals and the rules of biological safety were strictly observed [13].

When conducting cytogenetic studies, all operations when working with growth media and preparations were performed under sterile conditions using a laminar box. Buffers were prepared with double-distilled water, filtered through membrane filters (0.22 um «Millipor», Germany) and autoclaved at 1.2 bar for 30 minutes. Before use, glassware is pre-sterilized at 1600C for 120 minutes. Equipment, fixtures, utensils made of polymeric materials were exposed to ultraviolet light for 30 minutes.

Bone marrow cells were isolated according to a standard technique from the femur bones of animals sacrificed using a device for euthanasia.

Direct method. Red bone marrow was washed out of the femur with a nutrient medium with 0.04% colchicine into a centrifuge tube and incubated for 2-2.5 hours in a thermostat at 37 ° C. Then it was further incubated with a hypotonic solution of KS L for 40 minutes in a thermostat at 37 0 C. After hypotonization, it is treated three times with a fixative in the proportion of one part of glacial acetic acid and three parts of 960-1000 ethyl alcohol. The resulting precipitate was applied to a previously cleaned defatted glass slide and stained with Giemsa dye.

Method according to Ford. After the end of the drug administration, all the animals were injected intraperitoneally with 0.5 ml of a 0.1% colchicine solution, after 2 hours the animals were killed in strict accordance with the ethical principlesof working with laboratory animals. Red bone marrow flushed from a femur hypotonic solution COP L and incubated for 40 minutes in an oven at 37 0 C. After hypotension, they were treated three times with a fixative in the proportion of one partof glacial acetic acid and three parts of 960-1000 ethyl alcohol. The resulting precipitate was applied to a previously cleaned defatted glass slide and stained with Giemsa dye.

Statistical processing was carried out by the generally accepted methods of variation statistics.

All laboratory animals were divided into the following groups:

1 – group – white outbred rats (n = 12), receiving GMO soy with food for 30 days at 0.02-0.03 grams per one laboratory animal;

Group 2 – white outbred rats (n = 12), who received a standard diet and conventional soy.;

Group 3 – white outbred rats (n = 6), who received a standard diet without GMO soy and conventional soy.

As a GMO product in experiments used to I grown overseas and imported into our country only to perform scientific research of their work (see Picture 1).

Picture 1 – Appearance of GMO soybeans

Using the method of polymerase chain reaction (PCR), the presence of the 35 S + FMV promoter in the studied GMO- soy was revealed, which proves that the studied soy is a GMO-product. [15].

Cytogenetic changes in rat bone marrow cells were studied using the following methods: Direct method. Red bone marrow was washed out of the femur with a nutrient medium with 0.04% colchicine into a centrifuge tube and incubated for 2-2.5 hours in a thermostat at 37 ° C. Then it was further incubated with a hypotonic solution of KS L for 40 minutes in a thermostat at 37 0 C. After hypotonization, it is treated three times with a fixative in the proportion of one part of glacial acetic acid and three parts of 960-1000 ethyl alcohol. The resulting precipitate was applied to a previously cleaned defatted glass slide and stained with Giemsa dye.

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Results and Discussion

For the analysis, cells of the red bone marrow were used, in which elements of the mitotic apparatus were detected. Metaphase plates – this cells in which chromosomes are in the metaphase mitotic (somatic cell division stage). The number of chromosomes in rats is normally 42 (diploid set).

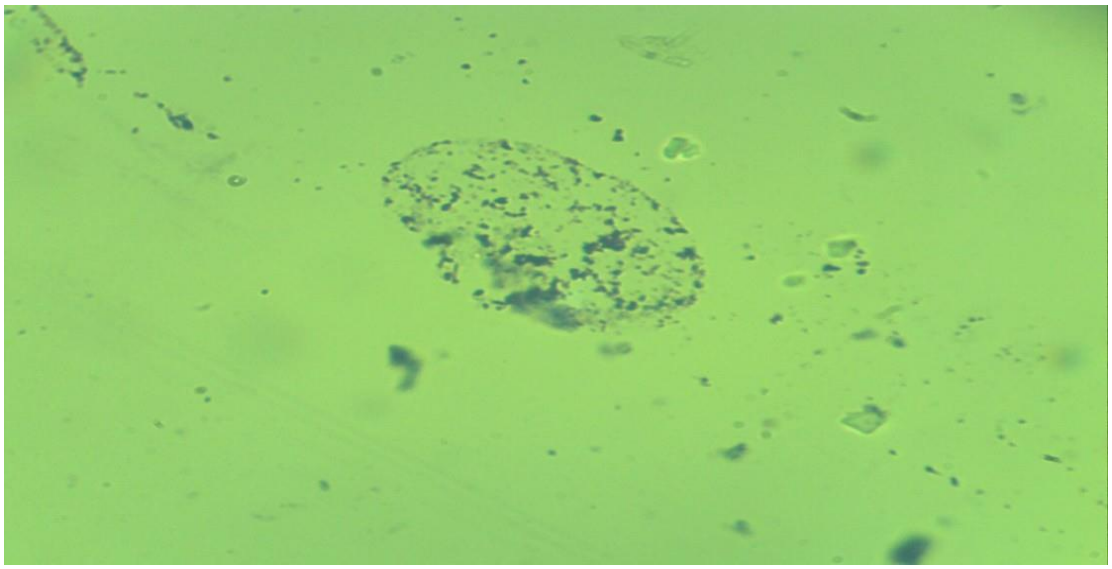
Analysis revealed that – the low mitotic activity of bone marrow cells in 1 – group (treated with GMO -soy) only at part in the bone marrow samples detected metaphase plate (Table1).

Table 1 – Indicators of cytogenetic changes in bone marrow cells of laboratory animals treated with a GMO product (soy)

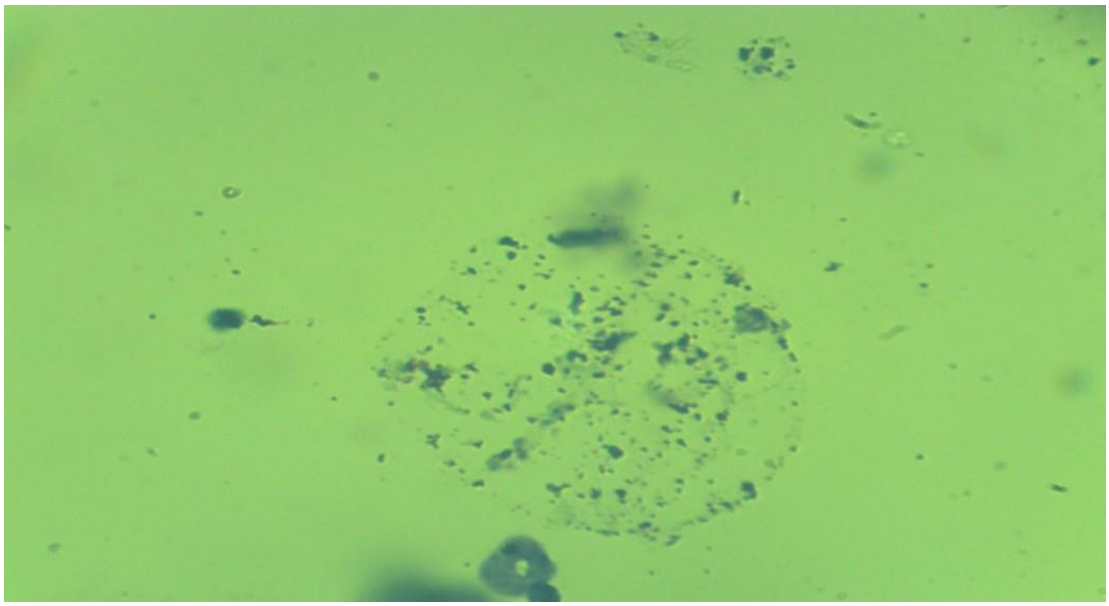
Group	Types of aberrations		
	Polyploidy	Aneuploidy	Structural changes in chromosomes
1 – group	5,6%	5,6%	Dispersion, Pulverization, Delayed mitosis in prophase
2 – group	Lack of	Lack of	Pulverization of chromosomes, K-mitosis
3 – group	Lack of	Lack of	Lack of

Of all the metaphases to be analyzed in laboratory animals of the 1st group, in 11.2% cases, polyploid (the cell contains a set of chromosomes more by a multiple than normal; 5.6%) and aneuploid (cells contain the number of chromosomes less or more than a multiple haploid set; 5.6%) karyotypes.

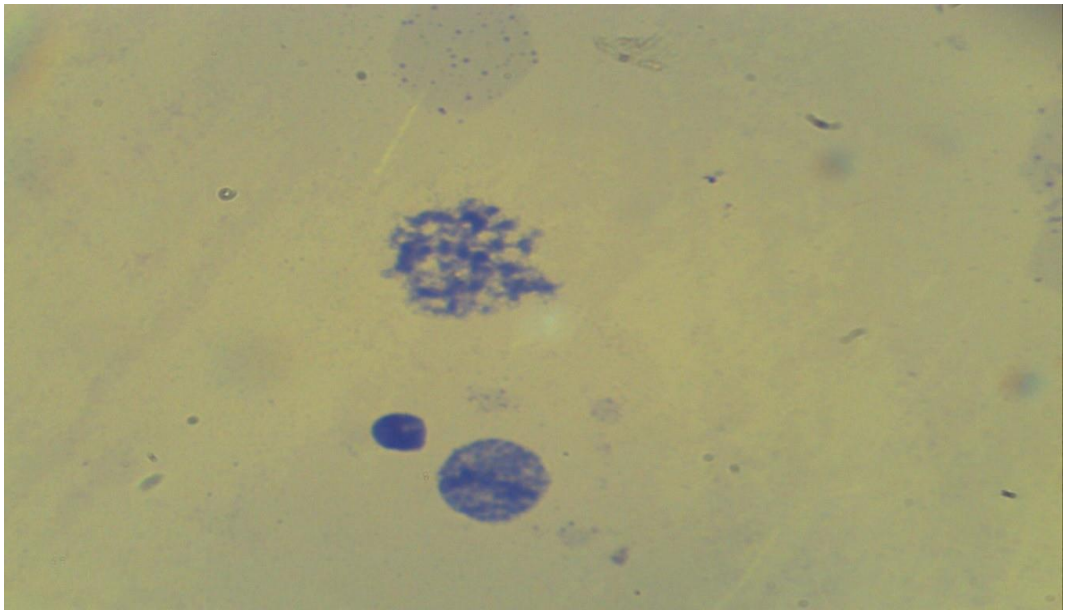
In other bone marrow samples from rats of this experimental group, metaphase plates were absent, but there were blast cells (cells at the stage preceding mitosis) and cells with mitotic pathology: chromosome pulverization (Picture 2,3), impaired chromosome spiralization and despiralization (Picture 4),premature spiralizationof chromosomes (Picture 5), delayed mitosis at the prophase stage. The rest of the metaphase plates contained a normal (42 chromosomes) karyotype.



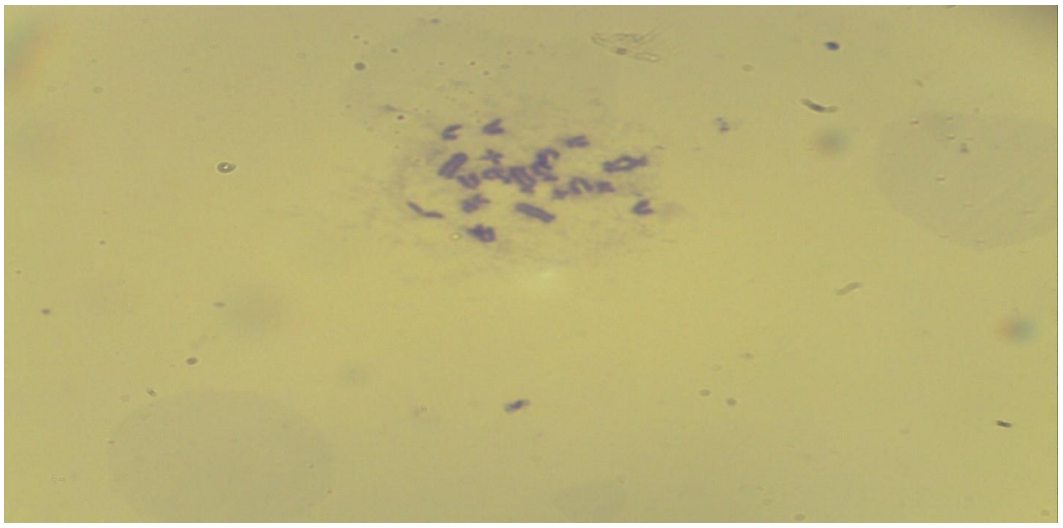
Picture 2 – Bone marrow cells of laboratory animals treated with GM-soy (group 1). Pulverization of chromosomes (approx. X 10, vol. X 100)



Picture 3 – Bone marrow cells of laboratory animals treated with GM-soy (group 1). Pulverization of chromosomes (approx. X 10, vol. X 100)



Picture 4 – Bone marrow cells of laboratory animals treated with GM-soy (group 1). Violation of spiralization and despiralization of chromosomes (approx. X 10, Ob. X 100)

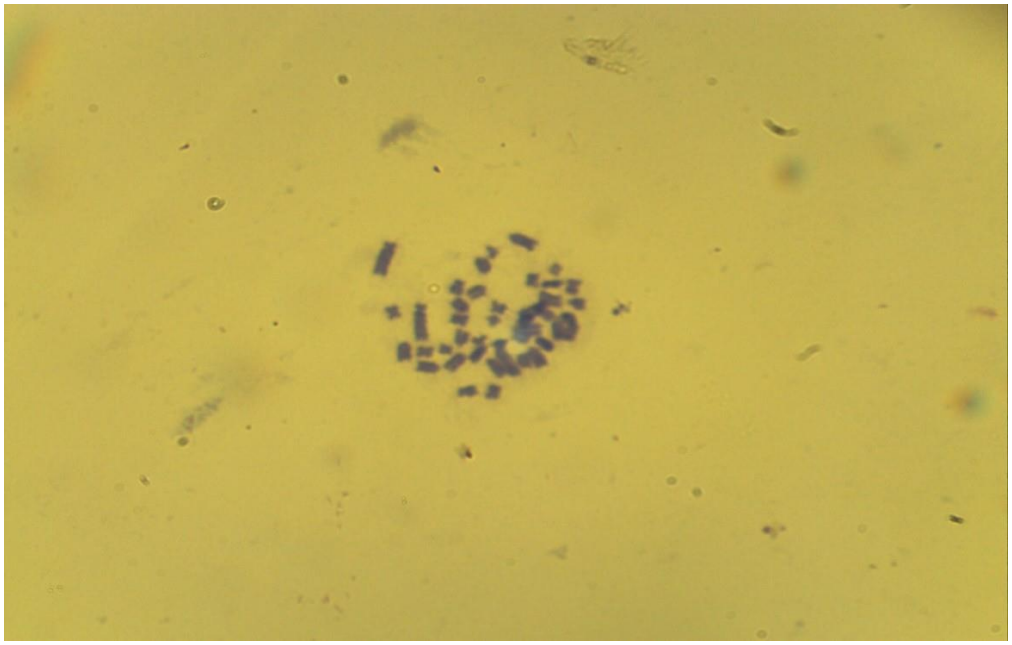


Picture 5 – Bone marrow cells of laboratory animals treated with GM-soy (group 1). Premature spiralization of chromosomes (Approx. X 10, Ob. x 100)

Experimental studies on laboratory animals proved that the used GM product (soy) led to cytogenetic changes in the cells of highly proliferating tissues, such as the red bone marrow of white outbred rats.

There is a partial inhibition of the proliferation of bone marrow cells due to the cytotoxic action, which can lead to cytopenia.

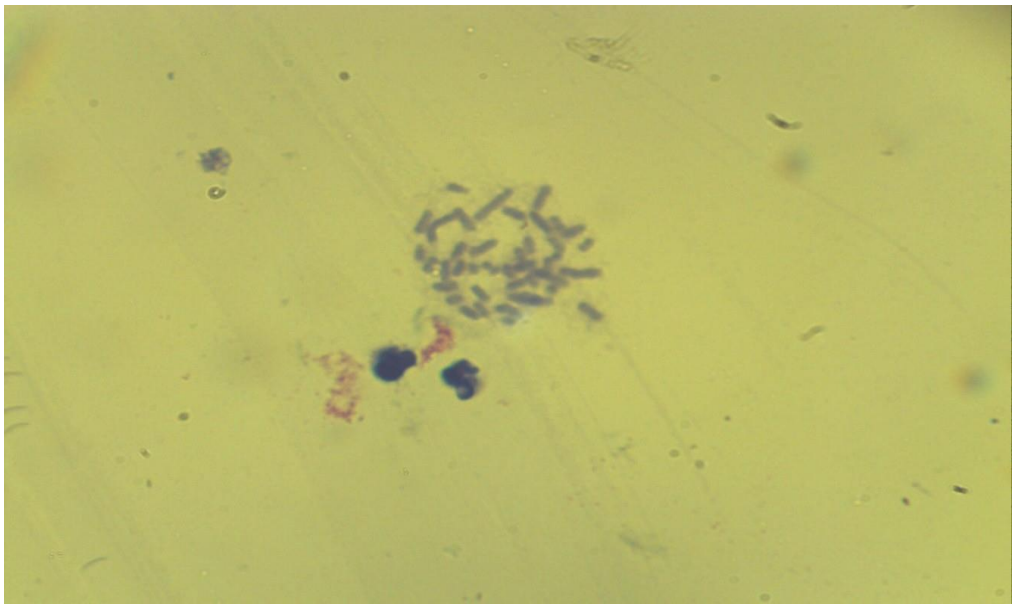
In group 2, polyploid (polyploidy) andaneuploid (aneuploidy) karyotypes were notdetected in only 50% of laboratory animals. The number of metaphase plates found in the bone marrow was the same with the animals of group 1. In all analyzed metaphases, the karyotype was not changed (Picture 6).



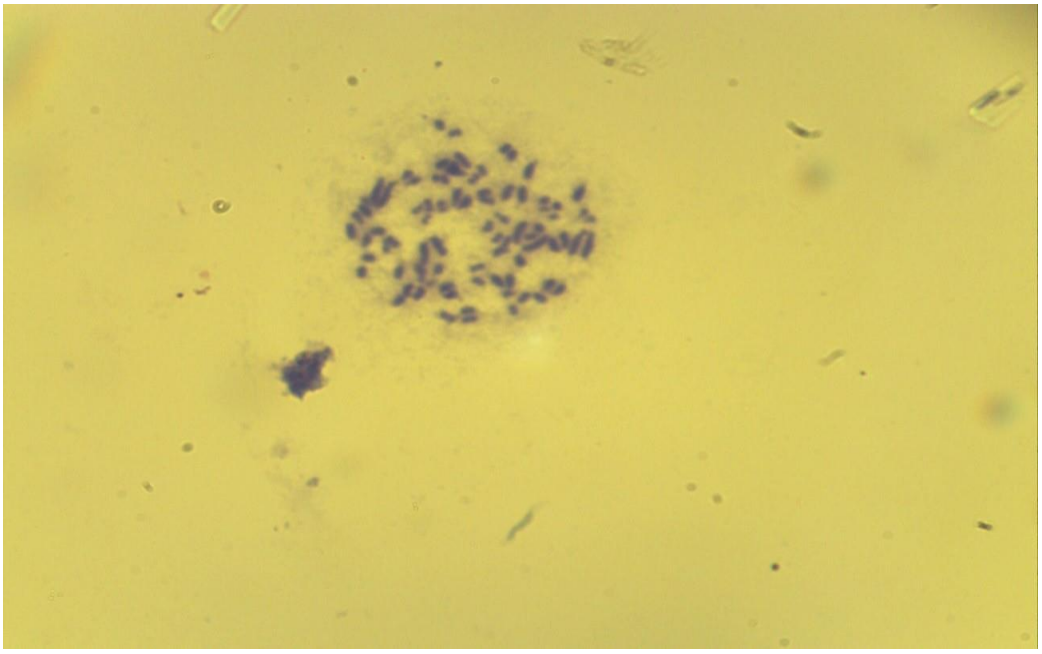
Picture 6 – Bone marrow cells of laboratory animals that received soy without GM (group 2). Normal karyotype (approx. X 10, vol. X 100)

It was found that the cells did not contain genomic abnormalities, all metaphases had a normal karyotype (42 chromosomes). In the remaining (50%) bone marrow samples, cells with mitotic pathology (chromosome pulverization, K-mitosis) were present, as well as in the bone marrow cells of group 1 outbred white rats.

In group 3 (control group), all bone marrow samples contained only metaphase plates with a normal karyotype (Picture 7– early metaphase;Picture 8– late metaphase).



Picture 7 – Bone marrow cells of laboratory animals that received a standard diet (group 3 – control). Normal karyotype, early metaphase. (Approx. X 10, Ob. x 100)



Picture 8 – Bone marrow cells of laboratory animals fed a standard diet (group 3 – control).
Normal karyotype, late metaphase (approx. X 10, vol. X 100)
The mitotic activity of rat bone marrow cells was higher than in the experimental groups (groups 1 and 2).

Conclusions

1. As a result, in the group of white mongrel rats treated with GM soybean detected cytogenetic changes in their red om bone ohm brain e lead partially m the inhibition and proliferation of bone marrow cells by the cytotoxic action, which may lead to cytopenias.

2. Of all be analyzed metaphases in laboratory animals Group 1 at 11, 2 % cases were identified altered karyotypes – polyploidy (5.6%) and aneuploidy (5.6%). In the remaining groups (group 2 and 3), no such changes were observed.

3. Bone marrow samples from 1-group rats contained blast cells and cells with mitotic pathology: chromosome pulverization, chromosome spiralization and despiralization disorders, premature chromosome spiralization, mitosis delay at the prophase stage.

4. In the group of laboratory animals as feed given to w without GM (group 2) was found to decrease the proliferation of bone marrow cells (at 50%), the rest in the bone marrow were present metaphase plate in all metaphases analyzed (36) the karyotype was not changed. The cells did not contain genomic abnormalities; all metaphases had a normal karyotype (42 chromosomes).

5. In the group of animals that did not receive soy with and without GM (3 – groups a control group), all bone marrow samples contained only metaphase plates with normal karyotype, and the cells did not contain genomic abnormalities.

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