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EVALUATION OF THE MUTAGENIC PROPERTIES OF THE FUROCOUMARIN EXTRACT FROM THE CELL CULTURE OF *CONIUM MACULATUM L.*

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The study presents data on the genotoxicity study of standardized by the amount and ratio of furocoumarins extract from the cell culture of Conium maculatum. The extract containing furocoumarins in the ratio: isopimpinellin - 42.97%, bergapten - 35.18% and xanthotoxin - 15.41%, in experiments demonstrated pronounced antithrombotic, myelo- and hepatoprotective effects under conditions of chemotherapeutic aggression - the introduction of the maximum tolerated dose of cisplatin. The extract is positioned as a promising herbal remedy for the relief of chemotherapeutic complications. The need to assess the genotoxicity of the presented composition and ratio of furocoumarins is primarily determined by ambiguous information on their effect on the genetic apparatus in various test systems. In vivo, the effect of a single and course intragastric administration of a standardized extract of a cell culture of Conium maculatum containing the amount of furocoumarins (isopimpinellin - 42.97%, bergapten - 35.18% and xanthotoxin - 15.41%), at doses of 30 and 150 mg / kg was studied. A cytogenetic analysis of the metaphase plates of the bone marrow of male and female CBA mice was carried out taking into account the number of damaged metaphases, the number of aberrant chromosomes, single fragments of chromosomes and polyploid cells in % per 100 cells. A 1% starch suspension was used as a negative control. Prior to the studies on D. Melanogaster, the dose of the investigated furocoumarin extract was determined by the survival rate of P1 females (wild type), which, at the maximum dose used, should not be less than 50%. The mutagenic activity of the extract was studied by somatic recombination (mosaicism) in D. melanogaster using marker mutations yellow and singed on 1000 females at a dose of 150 mg / kg. Thus, it was determined that the use of a Conium maculatum cell culture extract containing furocoumarins in the ratio: isopimpinellin - 42.97%, bergapten - 35.18%, and xanthotoxin - 15.41%, does not induce genetic damage in CBA mice and D. melanogaster, which is one of the objective criteria for the safety of its use. The results obtained determine the possible prospects for continuing research in terms of developing a new herbal medicinal product.

Keywords: chromosome aberrations, recombination, genotoxicity, furocoumarins.

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A wide range of pharmacological effects of pure furocoumarins and their combinations (antioxidant, anti-inflammatory, antiproliferative, gonadotropic and choleretic effects, the ability to modulate various biochemical pathways, use in dermatology, etc.), draws attention to this group of substances as a potential herbal medicines [7 , 11, 12, 13, 14]. At the same time, their chemical diversity, and more than 50 natural furocoumarins (FC) and a significant number of their combinations are known, when obtained from plant raw materials, complicates the search for the target efficiency of substances [8, 10]. In this regard, it seems promising to obtain furocoumarins standardized in terms of quantity and ratio from cell cultures of *Conium maculatum* (hemlock spotted). It is from the extract from *Conium maculatum* cell cultures containing furocoumarins (isopimpinellin - 42.97%, bergapten - 35.18% and xanthotoxin - 15.41%) that data on pronounced antithrombotic, myelo- and hepatoprotective effects have been obtained, which determines the prospects for its use in conditions of chemotherapeutic aggression [5]. At the same time, it is known that, depending on the chemical structure of the FC molecule, on the number and nature of substituent radicals in the compound, on the arrangement of cyclic systems (angular or linear - the bond of the furan ring with coumarin), as well as on the combinations and concentrations, a number of FCs have genotoxic effects [1, 6, 10, 15]. However, information on genotoxicity is not of a systemic nature, since the results of studies conducted *in vivo* or *in vitro* vary depending on the test systems used, doses and duration of use [7, 15]. The study of the genotoxicity of compounds, including those of plant origin, is the most important preventive measure to identify substances potentially dangerous to humans and their heredity [1].

Based on this, the aim of this work was to study the genotoxicity of the hemlock spotted cell culture extract in vivo in the bone marrow cells of CBA mice and somatic cells of *D. melanogaster*.

Material and methods. The study was carried out on 40 male and female CBA mice weighing 18-25 g. The animals were divided into groups: 1st group - males (n = 5) control for a single injection; Group 2 - males (n = 5), study of the effect of the maximum dose of the FC extract of 150 mg / kg with a single injection; Group 3 - males (n = 5) study of the therapeutic dose of FC 30 mg / kg with a single administration; 4th and 5th groups, males and females (n = 5), who received FC extract 30 mg / kg for 5 days; 6 and 7 groups - males and females (= 5) controls for groups 4 and 5; Group 8 positive control - a single injection of cyclophosphamide (CP) at a dose of 20 mg / kg. CP is a classic mutagen and is used in laboratory studies [3]. The choice of the dose of the extract was determined by the literature data, as well as the results of preliminary studies [5]. Control animals under similar conditions were injected with 1% starch suspension in an equivalent volume. All manipulations with animals were reviewed by the local commission for the care and use of animals for compliance with regulatory acts.

The extract was obtained by the method described in the patent of the Russian Federation No. 2713118 [2]. The qualitative composition of FC was determined using gas chromatography — mass spectrometry (GC-MS) and thin layer chromatography (TLC), quantitative content and purity were determined by high performance liquid chromatography (HPLC). The standard samples of bergapten, xanthotoxin, and isopimpinellin were used (Sigma-Aldrich, USA).

Analysis of chromosomal aberrations in metaphases in vivo takes into account factors such as absorption, distribution, metabolism and excretion, which makes it highly informative and accurate [3]. Bone marrow (BM) chromosome preparations were prepared according to the modified Ford method, stained with azure II-eosin for 40 minutes. Cytogenetic parameters (the number of damaged metaphases, the number of aberrant chromosomes, polyploid cells in% per 100 cells) were assessed 24 hours after the last injection. The analysis included 100 BM metaphase plates from one animal (per group of 500). Statistical analysis was performed using StatPlus Pro [Build 6.7.1.0.]. For each sample, the arithmetic mean (X) and the arithmetic mean error (m) were calculated. The nonparametric Mann-Whitney test was used. The significance level of the criteria was set equal to 1% and 5%.

The classical method of testing for mutagenicity is the method of somatic recombination (mosaicism) in *D. melanogaster* using the *yellow (y)* and *singed (sn)* marker mutations (SMART test) [3]. At the same time, marker recessive mutations of the parents are in a homozygous state, the parents have the corresponding phenotype - yellow females and curly males. When such individuals are crossed in the offspring, the genes pass in the ysn + / y + sn transposition, and due to heterozygosity they do not appear phenotypically, the females in this offspring have a gray body and wings, the body is covered with normal hairs and bristles. When disorders appear in the chromosome, in particular under the influence of various external influences, the genes go into a homozygous state, which leads to a phenotypic change in the shape and color of the bristles. It is believed that the main mechanism for the occurrence of spots is somatic crossing over, the frequency of which can serve as a measure of the effect of mutagens. Depending on the place of chromatid rupture in females, spots of various types appear on the body. The gap between the *sn* gene and the centromere results in the formation of a *yellow-singed* double spot. The gap between the *sn* and *y* genes, as well as double crossing over lead to the appearance of single spots (*y*) and (*sn*), respectively [9].

After 48 hours, the investigated FC extract was added to the nutrient medium, where the eggs were already laid, at a dose of 150 mg / kg. After 9-10 days, the hatched females were examined under a stereoscopic microscope.

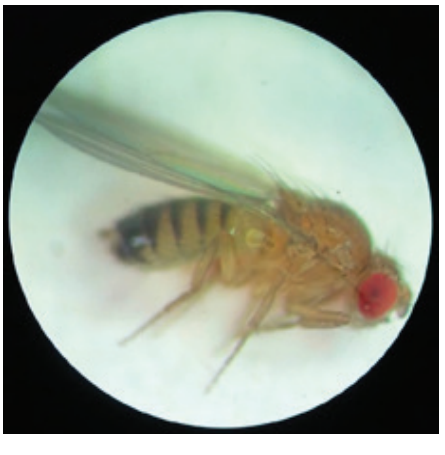
The choice of doses for *D. melanogaster* differs from the choice of doses for mammals. The rationale for the dose used is to conduct a study on the survival of P1 females (wild type), which, at the maximum dose used, should not be less than 50%. [3]. FC doses of 50, 100, and 150 mg / kg were used. In test tubes with a dose of 150 mg / kg, the survival rate of females was more than 50% compared to the control, which indicates the possibility of testing it in this experiment. The significance of differences for the rate of occurrence of females with mutations during statistical processing was assessed using the χ^2 test (chi-square) with Yeits's correction, which is used only for 2 × 2 tables. For a 5% significance level, the critical value of χ^2 is 3.84 [4].

Results and discussion. In animals of the 2nd group, with an exposure of 24 hours, 1.80 ± 0.73% of aberrant metaphases were revealed. All structural changes in chromosomes were single fragments and gaps. The number of aberrations did not change in comparison with the same indicator in the control (group 1). In group 3, 1.00 ± 0.32% of cells with chromosome aberrations were found. Structural disorders are represented by single fragments and gaps, which corresponds to the values of the control. In the 8th group - positive control - the appearance of metaphase plates with aberrant chromosomes is induced in BM, and the proportion of damaged metaphases is 13.4 times higher than that of the 2nd group and 24 times higher than those of the 3rd group. The proportion of damaged metaphases in the 8th group is significantly higher than that of the 1st group. The number of aberrant chromosomes in group 8 reached 45.80 ± 3.76%. Damage was represented by single and paired fragments, exchange violations, gaps, the content of which significantly exceeded those of the 1st, 2nd and 3rd groups.

After the course of administration of FC in the BM of males (4th group) 2.75 ± 0.63% of aberrant metaphases were detected, in females (5th group) the same indicator was 2.20 ± 0.37%. Chromo-

a

b



Phenotypic manifestation of the singed mutation singed bristle - A; D. *melanogaster* wild type, normal bristles - B.

some aberrations were represented by single fragments and gaps, the number of which corresponded to the level of control values.

Thus, a single and course intragastric administration of an extract of a cell culture of *Conium maculatum* to male and female CBA mice does not change the proportion of damaged BMCs, does not increase the number of aberrant chromosomes and gaps in comparison with the negative control, i.e. does not have a clastogenic effect on chromosomes.

As a part of the study of the mutagenic properties of FC in vivo, the SMART test for *D. melanogaster* was carried out.

Analysis of females that flew out showed that the extract of hemlock did not induce the appearance of mutant spots in them, compared with those in the control. For 1000 examined females, 5 individuals with a singed seta "*sn*" were identified, the χ^2 value was 0.51 (<3.84), which is not a statistically significant change. Females bearing single "*y*" spots and double "*sn*" spots were not identified. Thus, FC at a dose of 150 mg / kg is not genotoxic in this test, does not increase the number of recombination and mutational events in somatic cells of Drosophila larvae after exposure.

In this study was used an extract from a mixture of furocoumarins, most of which is represented by isopimpinellin - 42.97%. The presence of two methoxy groups in the molecule leads to the loss of photosensitizing activity [6]. In the literature, DNA damage by furocoumarins is associated with a photosensitizing effect, because under the influence of light DNA covalent bonds with intermolecular complexes and the spatial structure of nucleic acids changes, and photoinactivation of enzymes occurs due to the oxidation of amino acid residues [6, 16]. The predominance of isopimpinellin, which does not have photosensitizing activity, in the studied extract, and the minimal content of the other two compounds, probably led to the absence of genotoxic action.

The genotoxic properties of plant compounds can undoubtedly depend on the method of obtaining plant substances, the extraction of their components [1]. The investigated extract was obtained according to the original method, its composition is standardized, which can provide the content of the indicated substances in the appropriate amount, and hence the stable effects from its use [2].

Based on the results of the study, it can be concluded that a single intragastric administration of FC at doses of 30 and 150 mg / kg, as well as a course administration of FC to male and female CBA mice for 5 days (30 mg / kg) does not affect the level cytogenetic abnormalities in the BM. In the test system of somatic recombination (mosaicism), when FC was added to the nutrient medium at a dose of 150 mg / kg, no changes in the frequency of appearance of mutant bristles and spots on the body and head were found in *D. melanogaster* when using yellow and singed markers. Consequently, the extract of the *Conium maculatum* cell culture does not cause genetic changes, which is one of the objective criteria for safe use.

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