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Original Contribution

INVESTIGATION OF THE CYTOGENETIC EFFECT OF THE INSECTICIDE KARATE ON RABBIT PERIPHERAL BLOOD LYMPHOCYTES

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ABSTRACT

The genotoxic effect of the insecticide Karate was investigated *in vitro* in rabbit peripheral blood lymphocytes. Lymphocyte cultures, obtained from three rabbit blood donors, were exposed to the action of Karate at four experimental concentrations. The cultures were treated with Karate on the 24th hour marking the beginning of incubation, and the exposure lasted up to the end of incubation, i.e. for another 24 hours. The cytogenetic analysis of results revealed a dose-dependent increase in the frequency of chromosomal aberrations, with maximum effect after exposure at 1.5µg/ml lambda-cyhalothrin. The presence of satellite associations and gaps, as well as of aneuploid cells in *in vitro* treated rabbit peripheral blood lymphocytes was detected.

Key words: mutagenesis, lymphocytes, rabbits

INTRODUCTION

During the last years, the toxicity of pesticides is studied by a number of authors not only with regard to their importance and wide application in agriculture, but also because many insecticides are proven to have carcinogenic, clastogenic and mutagenic activity (1, 2, 3, 4)

The active ingredient of the pesticide Karate – lambda-cyhalothrin, is a synthetic pyrethroid with a broad spectrum of insecticide and acaricide activity. Pyrethroids modify the conductivity of electrosensitive alkaline channels of neurons in mammals and invertebrates (5, 6). Lambda-cyhalothrin impairs the normal function of the organism, destroying the nervous system of insects and as a result, provoking paralysis or death (7, 8).

It was found that lambda-cyhalothrin is moderately toxic for mammals (9, 10, 11) and highly toxic for fish, aquatic invertebrates and bees and could cause death in these species at low concentrations and contact with them (12, 13). Similar to other compounds from the pyrethroid family, the toxicity of lambda-cyhalothrin could vary depending on both its concentration and the nature of used solvent (14). The absorption of lambda-cyhalothrin is evaluated via determination of its metabolites in urine and serum (15). Recently, the toxicity of the pesticide is extensively studied in insects and mammals, but the data about the genotoxicity and cytotoxicity are few (16, 17).

The studies on insecticide's toxicity showed that LD50 of lambda-cyhalothrin in rats was 56-79 mg/kg; the oral LD50 dose in honeybees was 38 mg/bee, whereas the contact LD50 was 0.9 µg/bee (18).

The hazard for action of the insecticide on populations that are not purposefully treated but could be influenced because of its wide use in agriculture, motivated the present study of the determination of the potential clastogenic and mutagenic effect of lambda-cyhalothrin in one biological species – the rabbit.

MATERIALS AND METHODS

The present investigation was conducted in order to test the mutagenic effect of the insecticide Karate. The active substance of the preparation was the synthetic pyrethroid lambda-cyhalothrin with the following characteristics (Figure 1):
Molecular formula: C23H19CIF3NO3,

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molecular weight: 449.9, chemical name: lambda-cyhalothrin - alpha-cyano-3-phenoxybenzyl-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate.

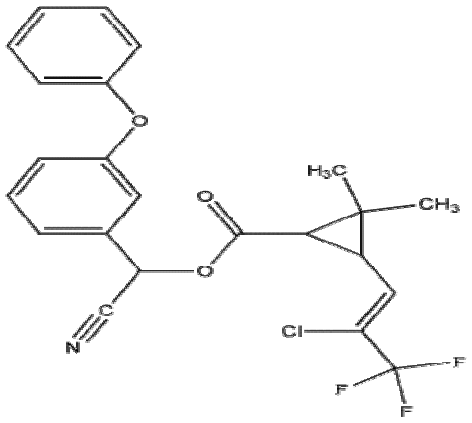

Figure 1. *Molecular structure of lambda-cyhalothrin*

Experimental animals

Female White New Zealand rabbits, aged 6 months, with equal body weight, housed and fed under the same conditions in individual cages, were used.

Preparation of lymphocyte cultures and pesticide solution

Blood samples were obtained from 3 blood donors from the marginal ear vein with 30 U/ml heparin as anticoagulant. Immediately after sampling, the blood was subjected to cytogenetic analysis in the following way (19): 0.5 ml whole heparinised blood was incubated in 7 ml RPMI 1640, 3 ml heat-inactivated normal calf serum, 0.2 ml reconstituted PHA, 100E/ml penicillin, and 50 µg/ml gentamicin.

The cultivation flasks were thermostated in the dark at 39^0 C. By the 24^{th} hour of the cultivation, Karate solution at the following concentrations was added:

- Group 1 – control, untreated cultures;
- Group 2 – treated with 0.6 µg/ml lambda-cyhalothrin
- Group 3 – treated with 0.9 µg/ml lambda-cyhalothrin
- Group 4 – treated with 1.2 µg/ml lambda-cyhalothrin
- Group 5 – treated with 1.5 µg/ml lambda-cyhalothrin

Each group included two cultivation flasks from each donor.

All cultures were incubated for another 24 hours. At the end of the 48^{th} hour from the beginning of lymphocyte incubation, chromosomal preparations for detection of chromosomal aberrations were prepared.

The Karate insecticide was dissolved in DMSO, with the final concentration of the agent being lower than 1% of the cell culture volume.

Chromosomal analysis

Chromosomal aberrations were determined after addition of colcemide at a final concentration of 0.5 µL/ml for fixation of cells at the metaphase stage (2 hours prior to end of incubation). The fixation was performed with methanol and ice-cold acetic acid (3:1). The cell suspensions were dropped onto slides and air-dried without slide warming.

Preparations were stained with the Giemsa stain.

Statistical analysis:

The data were statistically processed by the t-test for dependent samples (StaTistica software).

RESULTS AND DISCUSSION

A total of 1500 metaphase plates from three blood donors exposed to the influence of various concentrations of the insecticide lambda-cyhalothrin (Karate) were studied. The results of the chromosome analysis are presented on **Table 1**.

The analysis of results showed a lower frequency of gaps compared to satellite associations. Within the used dose range of treatments, rabbit lymphocyte cultures exhibited a dose-dependent increase of the frequency of induced gaps (**Figure 2**) after treatment at various doses of lambda-cyhalothrin. The maximum frequency of this type of aberrations was obtained following treatment at 1.5 µg/ml.

Similar results were obtained for satellite association numbers (**Figure 3**) and aneuploid cells. Satellite associations increased in a dose-dependent manner, with significant differences *vs* untreated controls at treatment with lambda-cyhalothrin levels of 0.9 µg/ml and higher

A significant increase in aneuploid cell number was registered only after treatment of lymphocyte cultures with 1.5µg/ml lambda-cyhalothrin $p<0,05$

The individual differences in the number of chromosomal aberrations in the three blood donors are presented in **Figure 4**. There were no significant differences in the frequencies of induced changes in peripheral blood lymphocytes in donors treated with

equal concentration and for an equal period with the insecticide p>0,05

Table 1. *Frequency of chromosomal aberrations in rabbit lymphocytes, treated with different doses of lambda-cyhalothrin.*

Lambda-cyhalothrin concentration, µg/mL	***Number of analysed metaphases***	***Metaphases with gaps, % x±SD***	***Metaphases with satellite associations, % x±SD***	***Aneuploid cells, % x±SD***
Control group	300	0	0.66±0.57	0.33±0.57
Group 1 – 0.6	300	2.0±1.0	2.66±1.52	0.66±0.57
Group 2 – 0.9	300	3.33±0.57[a]	4.0±1.0[a]	2.33±1.15
Group 3 – 1.2	300	4.66±1.15[a]	4.66±1.15[a]	3.0±1.0
Group 4 – 1.5	300	6.33±0.57[b]	7.0±1.0[a]	3.66±0.58[a]

Significant vs the control group: a – p<0.05; b – p<0.01,

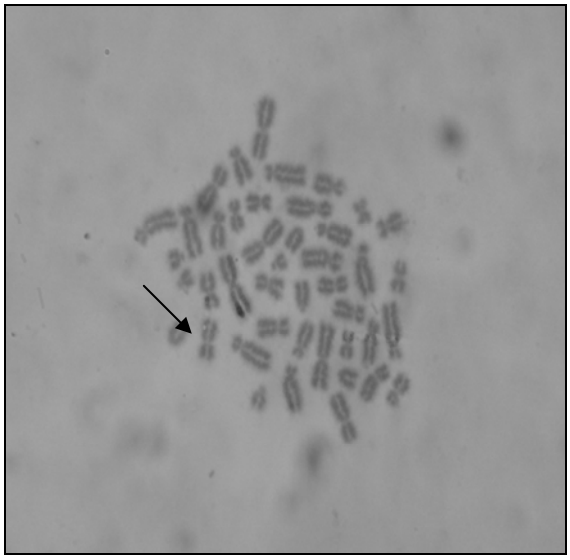

Figure 2. *Metaphase plate with a chromatid gap*

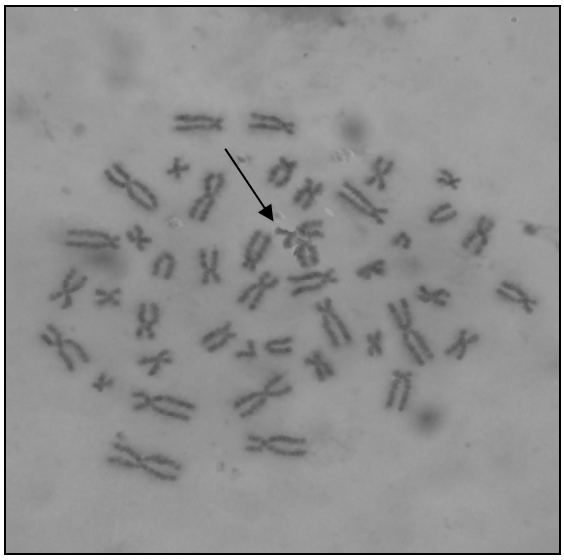

Figure 3*. Metaphase plate with satellite association*

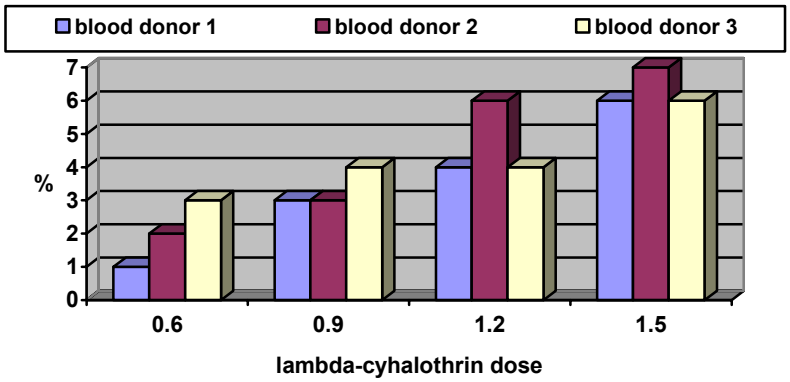

Figure 4. *Individual differences in the frequency of induced gaps.*

In the present study on the effect of subtoxic doses of lambda-cyhalothrin we determined an *in vitro* genotoxic effect of the preparation in rabbit lymphocytes. The concentrations of the pesticide used for a 24-hour treatment of cell cultures were considerably lower than the LD50 for human peripheral blood lymphocytes determined by Naravaneni, 2005 (20).

The observed dose-dependent frequency of damaged cells was in concordance with the data for genotoxicity of the preparation, tested in bone marrow cells of rats via the chromosomal aberrations and micronucleus tests (21). The genotoxic potential of lambda-cyhalothrin was also confirmed in fish (22) exposed to various concentrations (0.005-0.05 microgram/l) of the preparations for 36 hours.

According to some authors (23, 24) the

increase in the frequency of satellite associations after exposure to a number of mutagens is indicative for the increased probability for formation of chromosomal translocations. On the other hand, throughout the evaluation of the genetic risk, the increased frequency of chromosomal aberrations is related to higher risk of development of malignancies. Awa (25) detected a positive correlation between the risk of genetic diseases in populations and the level of cytogenetic damage whereas Au et al (26) hypothesised that chromosomal aberrations were in the background of carcinogenesis and that the determination of their incidence was an important parameter for the effect of various agents on the health status of mammals and man.

CONCLUSIONS

The obtained results of the chromosomal analysis provided evidence for the genotoxic potential of the insecticide Karate. The analysis of the effect of various doses of the preparation in cell cultures showed a dose-dependent increase in the proportion of induced aberrations in rabbit lymphocytes within the studied dose range. The nature of DNA aberrations induced by lambda-cyhalothrin suggested that they could be repaired, but this is a subject of future investigations.

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