

**PATHOLOGY OF TESTES CELLS IN WHITE MICE AFTER IMPACT OF EPRINOMECTIN**

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**ABSTRACT**

Avermectines are new class of antibiotics with acaricidal and anthelmintic activity. New medicine containing macrocyclic lactones is Eprimec. One milliliter of Eprimec contains 10mg of eprinomectin as active substance. The aim of our study was to determine the extent of karyopatic and cytotoxic effects of that preparation to cell division process in testes of white mice. The materials for the study were the non-linear white mice – males 18 - 22 g. Experimental group of mice received a dose of 200 micrograms of active compound per 1 kg of weight or 10 mg / ml of eprinomectin by single subcutaneous injection. The control group of animals remained intact. Each group consisted of 8 mice. The animals were slaughtered at 12 hours after drug administration. Testes of mice were isolated, smears – imprints were prepared that are fixed by May - Grunewald and stained with azure - eosin by Romanovsky. Glass slides with testes cells were viewed under a microscope and implemented to count the number of dividing cells, take into account the shape, size and color of the nuclei. Analysis of the frequency of occurrence of a karyopatic disorders and cytotoxic effects of cells were performed separately and lying sprawled cells with counting at least 1000 cells in each glass slide (Lazareva et al, 2016). Analyzing the data, we concluded that on the basis of eprinomectin formulations significantly reduce the activity of cell division, but it is toxic and cause serious karyopatic changes in the cells of the testes nonlinear white mice, indicating that the negative effects on the reproductive system of males.

**Keywords:** *antiparasitic medications, prinomectin, karyopatic effects.*

**INTRODUCTION**

Works of plenty of scientists evidence the kariopatic effect of chemical substances and antigens ( lov, 1972; Tolbert et al., 1992; Morgan, 2007; Lazareva et al., 2016). Among the latest antiparasitic drugs a special place has avermectins are new class of antibiotics possessing insecticidal, acaricidal and antihelminthic effect. This pharmacological group of substances widely used for the treatment and prevention of parasitic diseases of agricultural, small domestic and exotic animals. Ivomec and its copy – Baymak, Sevmek, Bisection, Pandeks, Ivermectin, Everton,

Novomec are most popular for veterinarians. The popularity of products containing macrocyclic lactones of the following generations increases, in particular drug Eprimec. Eprimec includes as an active ingredient eprinomectin – 10mg/ml, as an auxiliary substances dimethylacetamide – 50 mg/ml, benzyl alcohol 10 mg/ml and triglycerides up to 1 ml. In appearance the drug is transparent solution of yellowish color. The main target actions are eprinomectin glutamylcysteine chloride channels and receptors of gamma aminobutyric acid in nematodes, the larvae of botflies and ectoparasites. The magnitude of the change of chloride ions current through the membranes of nerve and muscle cells disrupts the conduction of impulses, resulting in paralysis and death of parasites. Eprimec is a low-hazardous substances (4 hazard class according to GOST 12.1.007-76), in recommended doses has no embryotoxic, teratogenic and sensitizing effect; toxic to fish and bees. Data on the effects on the reproductive system of males in literature is absent.

The aim of our this study was to determine the extent of karyopatic and cytotoxic effects of that preparation to cell division process in testes of white mice.

### **MATERIAL AND METHODS**

Experiment was performed at the parasitology laboratory of Perm State Agricultural Academy in summer 2015. The materials for the study were the non-linear white mice – males 18 - 22 g. Experimental group of mice received a dose of 200 micrograms of active compound per 1 kg of weight or 10 mg / ml of eprinomectin by single subcutaneous injection. The control group of animals remained intact. Each group consisted of 8 mice. The animals were slaughtered at 12 hours after drug administration. Testes of mice were isolated, smears – imprints were prepared that are fixed by May Grunewald and stained with azure - eosin by Romanovsky. Glass slides with testes cells were viewed under a microscope and implemented to count the number of dividing cells, take into account the shape, size and color of the nuclei. Analysis of the frequency of occurrence of a karyopatic disorders and cytotoxic effects of cells were performed separately and lying sprawled cells with counting at least 1000 cells in each glass slide. To study the activity of cell division we used mitotic index (MI%) – the ratio of the number of dividing cells to the total number of cells that we see at the moment. Micronuclei were identified as rounded chromatin body with a smooth continuous edge, no larger than 1/3 of the nucleus, which was located separately from the main kernel, not refracted light, with the intensity of staining and pattern of chromatin as the basic core, and were in the same plane with the nucleus (Morgan, 2007). In addition, incorporate a dual-core cells, fragmentation and vacuolization core, early (premature) separation of chromatids in prophase, division of pathology associated with damage to the mitotic apparatus and a violation of cells division. For statistical data analysis we used the software package STATISTICA 6.

## RESULTS AND DISCUSSION

Data analysis of the experiment is shown in the table.

Table. The comparison between the control group and the group of animals exposed to the drug Eprimecto cells of the testes nonlinear white mice

Parameter	Control group	Experimental group
P /	11. 24±4. 75	5. 94±1. 29
I (%)	27. 87±3. 87	21. 21±5. 30
Path(%)	1. 85 ±0. 28	12. 86±4. 49

PM/ - prophase-metaphase/anaphase-telophase ratio

I(%) – mitotic index, the ratio of the number of dividing cells to the total number of cells

Path (%) – pathology of meiosis (%) in number of devising cells

In the control group we detected high activity of cell's division MI (%), and prophases and metaphases none significantly prevailed to anaphases and telophases. Lagging of chromosomes and groups of chromosomes in metaphase led among meiosis pathology, however number of pathologies remained in the limits of the physiological norm (Morgan, 2007).

In the experimental group of animals activity of cell's division in testes after injection of Eprimec decreased slightly, but amount of pathologic meiosis increased in seven times. At the same time disturbed the balance of the ratio of prophases and metaphases to ana- and telophases. This fact indicate about the interlocking of normal division is still in its early stages and the simultaneous production of pathological cells (Tolbert et al, 1992).

In the comparison to control group in experiment these abnormalities of meiosis, as agglutination of chromosomes, formation of anaphase bridges, premature chromosomes in prophase and metaphase, lagging chromosomes and groups of chromosomes at all stages of division appeared (Il'inskih, 1984). Detected pathologies are connect with destruction of chromosomes and mitotic apparatus that disrupt meiotic cytokinesis and delays, and as a consequence is likely to lead to cell death (Motorna, 2001; lov, 1972). In the experiment, we noted a significant number of cells with toxic vacuolization, and the appearance of cells with micronuclei.

## CONCLUSION

Analyzing obtained data; we came to the conclusion that drugs based on eprinomectin slightly reduce the activity of cell division, but cause serious toxic and variations changes in the cells of the testes nonlinear white mice that speaks about the negative effects on the reproductive system of males. The using of drugs based on eprinomectin requires further study, as well as strict control of veterinary specialists.

### REFERENCES

- Iov I. A (1972). *Citophysiology and pathology of mitosis*– M.: «Medicina», p. 264.
- Ильских N.N. (1984). *Cytogenetic analysis of effects of infection mutagenesis in connection with immunoreactivity of organism*. PhD thesis. <http://www.dissercat.com/content/tsitogeneticheskii-analiz-posledstvi-infektsionnogo-mutageneza-v-svyazi-s-sostoyaniem-immun#ixzz3LuKfOx7K>
- Lazareva, O.I., Sivkova, T.N., Tatarnikova, N.A., Patlusova, E.S. (2016). Changes in the testes of laboratory mice under the influence of somatic extract *Anisakis simplex*. *Perm Agrarian Journal*, N 4 (16), pp. 117-120.
- Morgan D. O. (2007). *The cell: principles of control*. New science press p. 297; ISBN 978-0-9539181-2-6.
- Motorna O. O. (2001). Analysis of lacI mutation in Big Blue transgenic mice subjected to parasite-induced inflammation. /O. O. Motorna, H. Martyn, G. J. Gentile, J. M. Gentile // *Mutat. Res. fund. And Mol. Mech. of Mutagen*. Vol. 484. pp. 69-76.
- Tolbert, P. E. (1992). *Micronuclei and other nuclear anomalies in buccal smears: methods development* / P. E. Tolbert, C. M. Shy, J. W. Allen // *Mut. Res.* 271. pp. 69-77.