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## Antiradical, Antimetastatic and Antitumor Activity of Kaolin Preparation “Kremnevit”

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*clay; Lewis lung carcinoma; antitumor activity; superoxide radical; neutrophils*

### Abstract.

**The objective** of the research was to determine antiradical, antimetastatic and antitumor activity of kaolin preparation “Kremnevit”.

**Material and methods.** Experiments were carried out on 30 male C<sub>57</sub>BL mice inoculated with Lewis lung carcinoma (LLC) cells. The rate of superoxide radical generation in tumor cells as well as in animal organs was determined using 1-hydroxy-2,2,6,6-tetramethyl-4-oxopyrrolidin- hydrochloride spin traps and electron spin resonance (ESR) spectroscopy at room temperature.

**Results** of the experiments showed that the preparation “Kremnevit” exhibits antitumor activity reducing the tumor mass by 24% in experimental animals compared to animals which did not receive the preparation. “Kremnevit” affects the rate of superoxide radical generation by intracellular (mitochondria) and extracellular sources (the NADPH oxidase of tumor-associated neutrophils (TANs)). The superoxide-generating activity of TANs increased in mice treated with “Kremnevit” compared to animals which did not receive it.

**Conclusions.** Non-toxic kaolin preparation “Kremnevit” has antitumor and antimetastatic activity regulating electron transport in hepatocyte mitochondria during superoxide radical generation by hepatocyte mitochondria and immunocompetent blood cells of mice inoculated with LLC cells.



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### **Problem statement and analysis of the recent research**

During the rehabilitation of patients with cancer after standard treatment there is a need to ensure the recovery process using the preparations of natural origin which regulate the metabolism, enhance the functional activity of body's systems, and increase the natural resistance of the human body. Clay is of special significance among drugs of natural origin. I.S. Chekman et al. [1, 2] studied the action of white clay (kaolin) as a remedy in skin diseases as well as an antacid in stomach ulcer and duodenal ulcer, acute and chronic hyperacid gastritis and food poisoning. In our previous works we studied pharmaceutical preparations "Benta" and "Kremnevit" that have successfully passed a sanitary inspection and were recommended for the usage. Previous studies using ESR spectroscopy demonstrated that these preparations contain a wide range of significant amount of paramagnetic centers which provide their biological activity [3]. In addition, pharmacopoeial blue clay preparation "Benta" was found to serve as an interceptor of superoxide radicals generated by both immunocompetent blood cells in patients with rectal cancer and parenchymal cell structures, adenocarcinoma of the rectum in particular [4]. The introduction of drug produced from montmorillonite-type clay "Benta" turned out to be promising for use in restorative procedures for patients with cancer in rehabilitation centers and health resorts.

**The objective** of the research was to study antiradical, antimetastatic and antitumor activity of kaolin preparation "Kremnevit".

### **Material and methods**

Experiments were carried out on 30 male C<sub>57</sub>BL mice inoculated with Lewis lung carcinoma (LLC) cells. The evaluation of the antitumor and antimetastatic activity and tumor transplantation were carried out in accordance with the protocol [5]. The rate of superoxide radical generation in tumor cells as well as in animal organs was determined using 1-hydroxy-2,2,6,6-tetramethyl-4-oxopyrrolidin- hydrochloride spin traps and electron spin resonance (ESR) spectroscopy at room temperature. A teaspoon of the preparation was diluted in 200 mL of distilled water and administered per os at a dose of 0.5 ml daily for 10 days. On the 20<sup>th</sup> day of the experiment the animals were sacrificed by decapitation, and the tumor and liver tissue were taken for study. The results were calculated using parametric statistical criteria. The results were presented as Mean±SD and considered statistically significant at P<0.05. The maintenance and manipulation of animals were carried out in accordance with "General Ethical Principles of Animal Experiments" approved by the first National Congress on Bioethics (Kyiv, 2001) and the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1985).

### **Results and discussion**

Kaolin preparation "Kremnevit" showed antitumor activity reducing the tumor mass by 24% in experimental animals compared to animals which did not receive the preparation. The decrease in the number of metastases in animals injected with "Kremnevit" was achieved. Among animals in the control group the average number of metastases per mouse and their volume constituted 6.8±3.1 and 159±54.2, respectively, while in animals of the experimental group these parameters were lower: 4.3±1.1 and 107±10.9, respectively (Fig. 1 and 2).

The preparation increased the rate of superoxide radical generation by hepatocyte mitochondria and the NADPH oxidase of tumor-associated neutrophils (TANs). The rate of superoxide radical generation by the NADPH oxidase of TANs was 1.01±0.31 nM/g wet tissue·min (mice which did not receive any preparation) and 1.10±0.22 nM/g wet tissue·min (mice which received "Kremnevit") (Fig. 3).

The superoxide-generating activity of the liver increased in mice treated with “Kremnevit” by  $2.65 \pm 0.12$  nM/g wet tissue·min compared to animals which did not receive it –  $2.13 \pm 0.27$  nM/g wet tissue·min (Fig. 4).

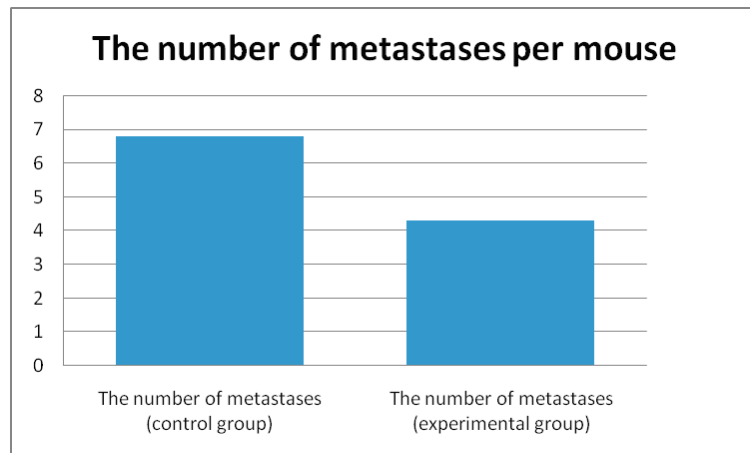


Fig. 1. The number of metastases per mouse

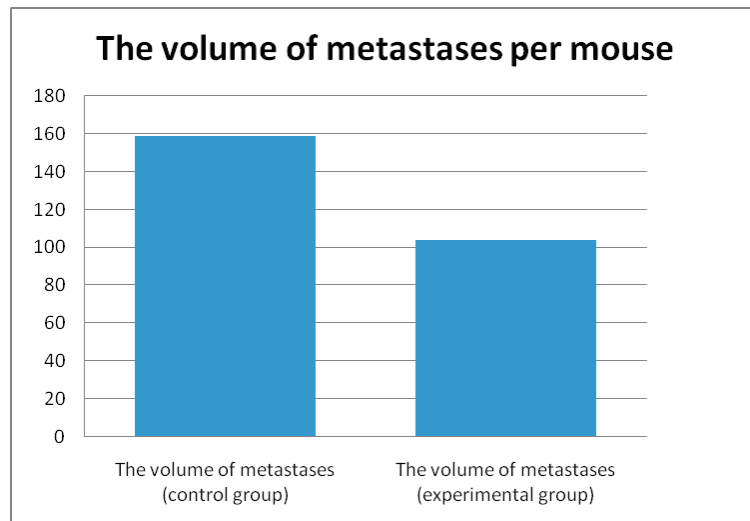


Fig. 2. The volume of metastases per mouse

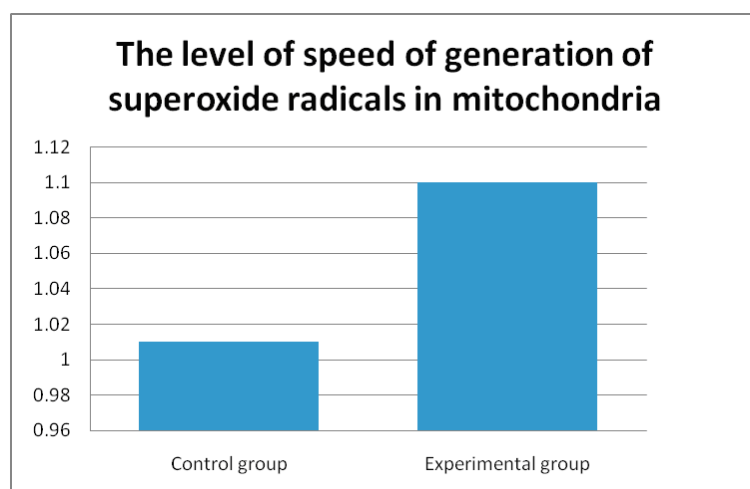


Fig. 3. The rate of peroxide radical generation in mitochondria

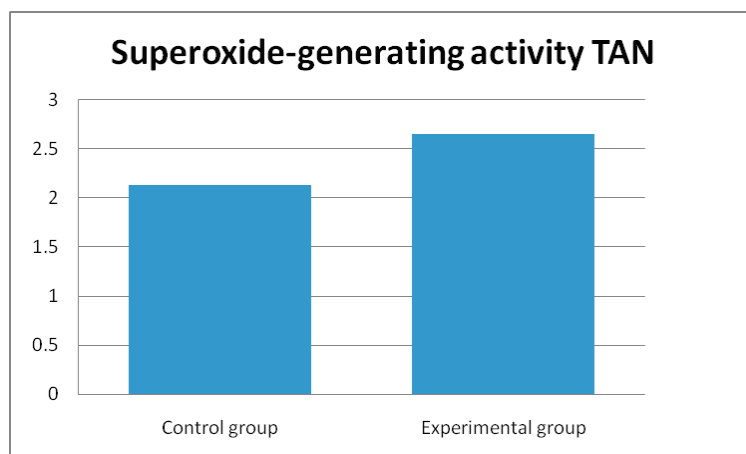


Fig. 4. Superoxide-generating activity of TANs

### **Conclusions**

New pharmacopoeial characteristics of kaolin preparation “Kremnevit” was discovered, namely its ability to influence electron transport during superoxide radical generation by hepatocyte mitochondria and immunocompetent blood cells of mice inoculated with LLC cells. Kaolin preparation “Kremnevit” exhibits antitumor activity reducing the number of metastases and the tumor mass in animals injected with “Kremnevit”. The introduction of pharmacopoeial kaolin preparation “Kremnevit” turned out to be promising for use in restorative procedures for patients with cancer after standard treatment in rehabilitation centers and health resorts.

### **Prospects for further research**

Antitumor and detoxification properties of kaolin preparation “Kremnevit” and the universality of the mechanism of its biological action are the basis for further in-depth study of the feasibility and effectiveness of its use as a drug support when applying various schemes of adjuvant and neoadjuvant chemo- and radiotherapy of patients with cancer.

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