



EVALUATION OF CYTOTOXIC AND CYTOSTATIC ACTIVITY OF *CHLOROPHYTUM COMOSUM* METHANOL EXTRACT

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ABSTRACT

Chlorophytum comosum leaf extract is well-known to contain flavonoids. The extract has cytotoxic and cytostatic activity in normal animal cells. At concentrations of 15 mg/mL and above, the extract leads to 100% death of SPEV cells. The number of dead cells of the SPEV line generally increases with increasing extract concentration. The LC50 of *Chlorophytum comosum* extract was determined by probit analysis (LC50 = 7.24 mg/mL). With an increase in the concentration of the extract, a noticeable decrease in the proliferative activity of cells was observed. After 48 hours, the inhibition of proliferative activity of SPEV cells is more pronounced than after 24 hours. The percentage of SPEV cells that died under the action of *Chlorophytum comosum* extract becomes smaller with increasing exposure time. The results of the analysis of the tumor mass at the end of the experiment fully correspond to the dynamics of its growth. The data obtained by us indicate the absence of the effect of *Chlorophytum comosum* extract against the transferable liver cancer of rats in an in vivo experiment.

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Introduction

Chlorophytum comosum belongs to poisonous plants due to the content of aristolochiic acid in the leaves [1]. However, in traditional medicine, this plant is still used to treat arthritis, facilitate childbirth, as part of weight loss supplements, treat rheumatism and stabilize the menstrual cycle, and as an antitumor agent [2-4]. However, there is a lack of scientific data on antitumor activity and its mechanisms [5, 6].

Recent studies show that *Chlorophytum comosum* extracts containing aristolochiic acid have pronounced carcinogenic and mutagenic effects [7, 8]. Therefore, the International Agency for Research on Cancer has classified *Chlorophytum comosum* as the first class of oncogenes that cause cancer in humans and animals [9]. The relationship between *Chlorophytum comosum* extracts containing aristolochiic acid and cancer of the upper urinary tract and kidneys was noted. Thus, about 1,500 genes with mutations, including mutations of the P53 gene, were found in malignant tumors in patients taking such extracts [10-12]. However, Rzhepakovsky *et al.* [13] developed a method for obtaining *Chlorophytum comosum* extract that significantly reduces its toxicity and, at the same time, provides a greater yield of flavonoids. Notably, other studies also revealed the presence of several positive pharmacological values in poisonous plant extracts containing flavonoids, including antitumor,

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anticachexic, anti-tuberculosis, anti-inflammatory, antipyretic, antimicrobial, and genoprotective activity [14-17]. According to Li *et al.* [18] leaves of *Chlorophytum comosum* contain such flavonoids as quercetin glycoside, quercetin (in hydrolysate), the flowers contain quercetin glycoside, and two flavonol glycosides. The data obtained earlier indicate the absence of toxicity and weak cytogenetic activity of the *Chlorophytum comosum* methanol extract [19].

Thus, this work aimed to study the activity of the flavonoid-containing extract of *Chlorophytum comosum* against the SPEV cells *in vitro* and to establish the presence or absence of its antitumor activity against transferable rat liver cancer *in vivo*.

Materials and Methods

An aqueous solution of dry methanol extract of *Chlorophytum comosum* leaves was used in this study. *Chlorophytum comosum* was grown in the laboratory greenhouse complex of St. Petersburg State Agrarian University (St. Petersburg, Russia). The extract was obtained by the method of Rzhepakovsky *et al.* [13].

The culture of pig embryo kidney SPEV cells was taken from the cryobank of the cell culture collection of the laboratory of the North Ossetian State Medical Academy (Vladikavkaz, Russia). The cell culture was grown in RPMI 4 medium (10% embryonic serum, gentamicin, ampicillin, amphotericin) in 6-well plates according to the method of Elati *et al.* [20]. The cells in the medium without the extract served as a control. An extract solution diluted in a nutrient medium with varied concentrations of 30, 15, 7.5, 3.75, 1.85, and 0.9375 mg/mL was introduced into six wells. Then, SPEV cells were cultured in a CO₂ incubator at 37 °C for 24 hours. Propidium iodide was used as a dye, which penetrates cells by destroying the membrane and binds to nuclear DNA [21]. To visualize the SPEV cells, a combination of several microscopic modes of light scattering and fluorescence detection on a Leica DM 2500 microscope, a Leica DFC 420C digital video camera with Leica Application Suite V 3.1 software was used.

Photographing was carried out in different modes: in phase contrast (allowing analysis of the surface of SPEV cells), at fluorescent illumination (allowing to count the number of dead SPEV cells by glowing red nuclei colored with propidium iodide), in normal light mode (allowing to visualize dead SPEV cells with living ones colored green with acridine orange) [22]. The following indicators were used in the experiment: the total number of SPEV cells in the field of view, the number of dead SPEV cells, and the number of living SPEV cells. Probit analysis was used to calculate the LC50 of the extract about SPEV cells [23].

An *in vivo* experiment was carried out in accordance with the Guide for the Care and Use of Laboratory Animals [24]. For the experiment, 12 male white laboratory rats weighing 150 ± 50 g were implanted subcutaneously in the scapula area with 0.5 ml of 25% tumor suspension in Hanks solution of the strain of alveolar liver cancer (PC-1), obtained from the bank of tumor strains of the North Ossetian State Medical Academy (Vladikavkaz, Russia). Animals with transferable cancer were randomly divided into two groups of 6 rats. In the experimental group, the solution was administered intramuscularly to rats at a dosage of 100 mg/kg, once a day for 16 days from the moment of tumor transplantation. After the withdrawal of the extract, animal observations continued for another week. The dynamics of tumor growth was assessed by changing its volume according to the Eq. 1 [25]:

$$V = A \times B \times C \quad (1)$$

where A is the width, B is the thickness, and C is the height of the tumor.

Measurements were carried out with an electronic vernier caliper every two days from the beginning of the experiment. On the 23rd day, the rats were removed from the experiment, and samples of organ tissue, tumors, and blood were taken for additional studies.

Statistical data processing was carried out in the STATISTICA 12.0 software using methods of biomedical statistics. The significance of differences in the parametric distribution was determined using the Mann-Whitney criterion for independent samples at $P < 0.005$ [26].

Results and Discussion

Investigation of the activity of a flavonoid-containing extract of *Chlorophytum comosum* against SPEV cells showed an interesting result. The control included living polygonal cells with 2-3 nucleoli. There was a visible uniform increase in the number of cells and the formation of a monolayer within 24 hours. The complete monolayer was formed in 48 hours. When exposed to the *Chlorophytum comosum* extract, compared with the control, there was a significant increase in the number of SPEV cells not attached to the substrate. Notably, the shape of SPEV cells under extract treatment changed from polygonal to rounded. Statistically significant differences in cell culture under the action of the extract were revealed at all concentrations compared with the control (**Table 1**).

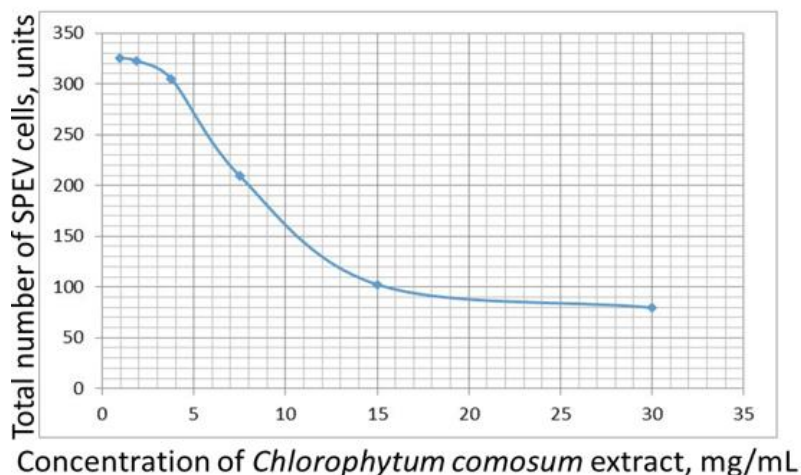
Table 1. Comparison of the number of SPEV cells in experimental groups

Experimental groups	Total number of SPEV cells, un.	Number of dead SPEV cells, un.	Number of living SPEV cells, un.
24 hours			

Group	24 hours	24 hours	24 hours
Control group	481.3±130.90	2.33±1.500	479.0±130.09
Experimental group (30 mg/mL)	79.75±72.06 *	79.75±72.06 *	0.0±0.00
Experimental group (15 mg/mL)	102.5±50.68	102.5±50.68 *	0.0±0.00
Experimental group (7.5 mg/mL)	209.5±74.77 *	114±48.9 *	95.5±31.45 *
Experimental group (3.75 mg/mL)	305.25±54.020	31.0±17.16	274.25±38.48 *
Experimental group (1.875 mg/mL)	322.75±38.600	38.25±26.110	31.0±27.30 *
Experimental group (0.9375 mg/mL)	325.75±77.070	41.25±29.680 *	284.5±53.03 *
48 hours			
Control group	461±110.9	9.0±9.11	452.0±109.66
Experimental group (1.875 mg/mL)	49±6.0 *	6.66±7.200 *	42.33±4.040 *

* P ≤ 0.005

According to **Table 1**, at ≥15 mg/mL *Chlorophytum comosum* extract, 100% SPEV cell death occurred after 24 hours. Under the action of *Chlorophytum comosum* extract, the total number of SPEV cells decreased at all concentrations (**Figure 1a**), which indicates the suppression of the proliferative activity of non-tumor SPEV cells [27]. With an increase in the concentration of *Chlorophytum comosum* extract, a noticeable decrease in the proliferative activity of cells was observed.



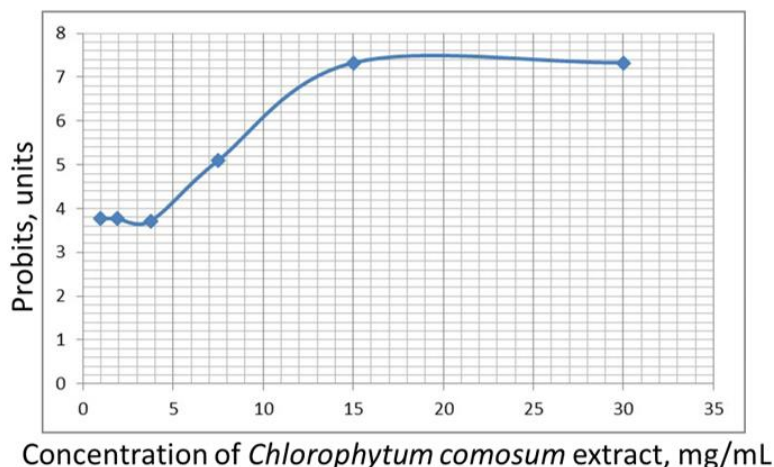
a)

Exposition time, h	Control group	Experimental group
24	481.3 ± 130.9	322.75 ± 38.6
48	461 ± 110.9	49 ± 6.0

b)

Figure 1. Assessment of the total number of SPEV cells: dependence of the total number of SPEV cells on concentration of *Chlorophytum comosum* extract (a) and effect of 1.875 mg/mL extract of *Chlorophytum comosum* on SPEV cells after 24 and 48 hours of exposition (b).

After 48 hours, the inhibition of the proliferative activity of SPEV cells was more pronounced than after 24 hours (**Figure 1b**). Interestingly, the number of dead SPEV cells generally increases with an increase in the concentration of *Chlorophytum comosum* extract (**Figure 2a**). The LC50 of *Chlorophytum comosum* extract was 7.24 mg/mL. It is worth noting, that the percentage of SPEV cells that died under the action of *Chlorophytum comosum* extract becomes smaller with increasing exposure time (**Figure 2b**).



a)

Exposition time, h	Dead SPEV cells, %
24	10.92
48	10.79

b)

Figure 2. Assessment of SPEV cells viability: dependence of the number of dead SPEV cells on concentration of *Chlorophytum comosum* extract (a) and influence of 1.875 mg/mL extract of *Chlorophytum comosum* on number of dead SPEV cells after 24 and 48 hours of exposition (b).

A study of the biological activity of the flavonoid-containing extract of *Chlorophytum comosum* in relation to transferable rat liver cancer *in vivo* revealed interesting results (**Figure 3**). In the control, a noticeable tumor growth was observed on day 11, which gradually increased until the end of the experiment. In the group of rats with an overgrown tumor under the action of the extract, tumor growth did not differ from the control until the 16th day of observation, and starting from the 17th day it became higher than in the control.

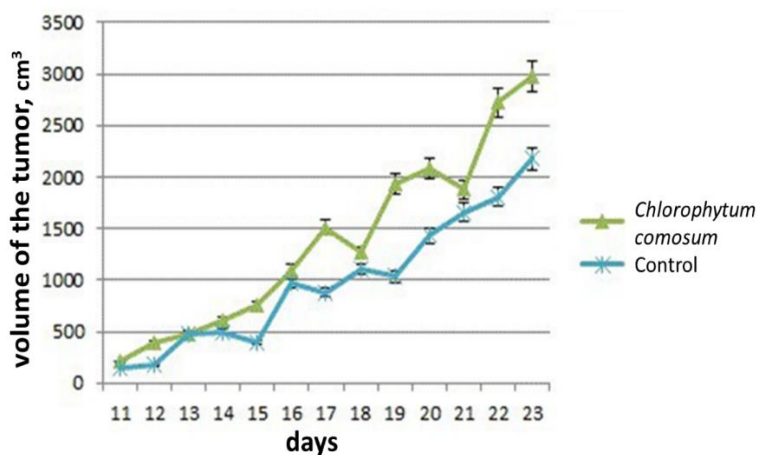


Figure 3. Dynamics of growth of the PC-1 transplanted tumor of rats under the action of *Chlorophytum comosum* extract.

The results of the analysis of the tumor mass at the end of the experiment fully correspond to the dynamics of its growth. The mass of the tumor practically did not differ from the control, which means that the extract we studied in the selected dosage does not have antitumor activity [28]. The data obtained by us indicate the absence of the effect of *Chlorophytum comosum* extract on the transplanted tumor PC-1.

Conclusion

Thus, the flavonoid-containing extract of *Chlorophytum comosum* does not have antitumor activity against PC-1 transferable liver cancer of rats and, considering the revealed cytotoxicity against non-tumor SPEV cells, it is likely to prove unpromising for further investigation of its antitumor activity against other types of tumors.

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Conflict of interest: None

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Ethics statement: The work with laboratory animals was carried out in accordance with the research protocol, which does not contradict the Geneva Convention of 1985 on "International Principles of Biomedical Research using Animals". The topic and descriptions of the experiments were approved by the Ethical Commission of the North Ossetian State Medical Academy (Vladikavkaz, Russia) (Protocol No. 13 of November 3, 2023).

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