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Exploring the Therapeutic Potential of Microscopic Fungi-Derived Substances Against Ehrlich Carcinoma: Insights from In Vivo Studies

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Abstract

Cancer remains a major global health challenge, necessitating continuous exploration of novel therapeutic strategies. The anticancer potential of intracellular biologically active substances NL-51, Mucor sp. M II-12, Penicillium Gz 9-10, and Gz 9-4 derived from microscopic fungi were investigated in Ehrlich's carcinoma-bearing lab. mice. Results showed that M II-12 significantly extended mouse lifespan and inhibited tumor growth compared to controls and other samples. Gz 9-10 and Gz 9-4 exhibited moderate effects, while NL-51 showed no favorable impact. The colorimetric assay revealed proteolytic activity in all samples. Although M II-12 additionally revealed inhibitory activity against proteolytic enzymes that support the cancer growth it does not inhibit the proteolytic enzyme present in tested M II-12 solution itself. It is believed that the synergistic action of these enzymes and inhibitors contributed to the observed anticancer effect. Conclusion. These findings demonstrated the anti-cancer potential of Mucor sp. M II-12. Further research focusing on elucidating the specific mechanisms underlying these effects could provide valuable insights for the development of novel therapeutic strategies against cancer.

Key words: microscopic fungi, cancer, Ehrlich carcinoma, protease, inhibitors.

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Introduction

Cancer is one of the leading causes of morbidity and mortality in the world. According to the WHO, cancer is a leading cause of death globally, accounting for around 9.6 million deaths in 2023. Cancer treatment is a multifaceted approach that aims to eliminate or control cancer cells, prevent their spread, and alleviate symptoms [1].

At present, despite of wide range of anticancer treatment methods (surgical intervention, chemotherapy, radiotherapy, immunotherapy, hormone-based therapy, etc.) [2,3] treatment still remains unsowed due to accompanying side effects (neuro-, hepato- and cardiotoxicity, superinfections) [4-7]. The high cost price of preparations is noteworthy also [8].

Consequently, researchers are actively exploring the development of more effective, safer, and affordable anticancer drugs and treatment approaches. Given the vast diversity of bioactive components present in microscopic fungi, these organisms emerge as promising candidates in the search for novel anti-tumor agents. Their rich contents of biologically active compounds offer a prospect of discovering new molecules capable of inhibiting cancer cell growth, inducing apoptosis, impeding angiogenesis, or enhancing immune responses against cancer. Harnessing the potential of fungal metabolites not only opens avenues for innovative treatment strategies but also holds promise for developing therapies with unique mechanisms of action, thus advancing the fight against cancer [9,10].

Among the diverse agents found in microscopic fungi, proteolytic enzyme inhibitors could be noteworthy. According to recent studies, in some types of tumors at an early stage of development, the level of proteolytic enzymes involved in the processes of cancer development (proliferation, angiogenesis, metastasis) is sharply increased. Therefore, their inhibitors could be considered as one of the most powerful anticancer treatment strategies [11,12].

The potential of protease inhibitors as powerful strategies to combat cancer points on their efficacy as promising anti-cancer treatments. Notably, these inhibitory compounds have been identified in microscopic fungi, further amplifying their potential as valuable candidates for the development of effective anti-cancer drugs. These findings emphasize the pivotal role that microscopic fungi can play in advancing therapeutic approaches for cancer treatment, opening new horizons in the ongoing pursuit of more efficient and targeted interventions against this devastating disease [13].

In addition to protease inhibitors, certain microscopic fungi also exhibit proteolytic enzymes with suppressive effects on tumor cells. Due to the variety of proteolytic enzymes, their functions may also vary significantly. In addition to some types of proteolytic enzymes promoting the development of tumors, there are proteolytic enzymes that, conversely, suppress tumor development. This diversity

underscores the complexity of proteolytic enzyme activity in cancer biology and the importance of understanding their specific roles in tumor progression or suppression [14,15].

Our research is focused on exploring the anticancer properties of intracellular biologically active substances derived from microscopic fungi, obtained through solid-phase fermentation, evaluate the efficacy of these substances in inhibiting the growth of Ehrlich carcinoma in laboratory mice and advance our understanding of the antitumor capabilities of microscopic fungi, contributing to the development of novel and effective therapeutic interventions against cancer.

Material and methods. Buffer salts including Na2HPO4, KH2PO4, NaCl, and KCl were procured from Alfa Chemical (India), meeting analytical grade standards.

The intracellular lysate of microscopic fungi (Fusarium sp.NL-51, Mucor sp. M II-12, Penicillium Gz 9-10, and Gz 9-4.), obtained through solid-phase fermentation, was sourced from the Scientific and Educational Center "Biomed" at the Technical University of Georgia. Ehrlich ascites carcinoma (EAC) cells were provided by the Kavetsky Institute of Experimental Pathology, Oncology, and Radiobiology of the National Academy of Sciences of Ukraine, Department of Experimental Cell Systems, from The Cell Line Bank (BCL) for Human and Animal Tissues (https://iepor.org.ua/www.onconet.kiev.ua).

Intracellular lysates were obtained through solid-phase fermentation of microscopic fungi.

Experiments were carried out on albino male mice weighing 20-25 g. Throughout the study, mice were housed under controlled environmental conditions with a 12-hour light-dark cycle at a temperature of 23±2 °C, receiving standard laboratory chow and unlimited access to water. For cancer modeling they were subcutaneously inoculated with a fixed number of viable cancer cells (2×10^6 cells/20 g body weight). Viability of cells was determined using the hemocytometer by trypan blue exclusion assay [16].

Experimental groups received 100 µl injections of test solutions (NL-50, M II-12, Gz 9-10) with a protein concentration of 1 mg/ml, while the control group received 100 µl of a physiological solution. The observation period spanned 52 days.

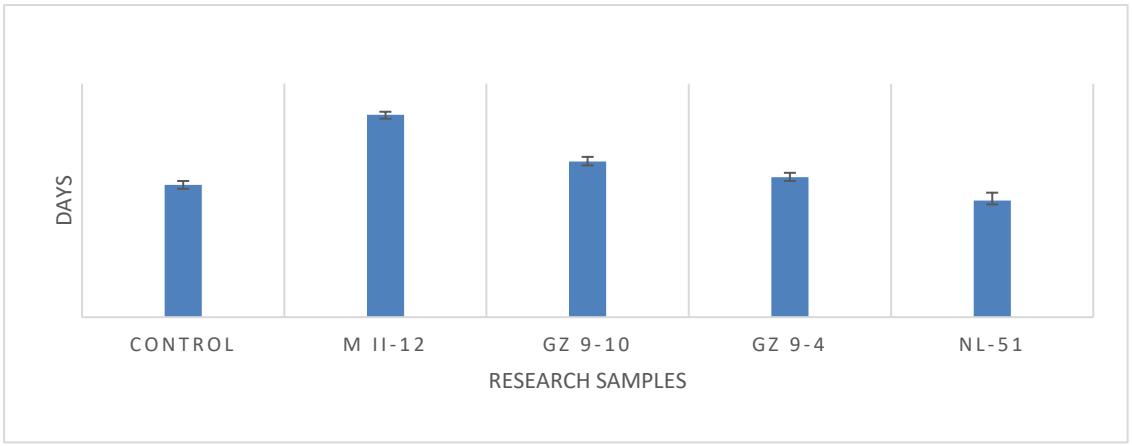
Tumor tissue volume was measured using a caliper. The tumor size was calculated using the formula $V=AxB^2x\pi/6$, where V is the tumor tissue volume, A - the length and B - width of tumor tissue [17].

Proteolytic activity and inhibitory effects of tested lysates isolated from microscopic fungi were determined using a colorimetric method [18].

Data analysis was performed using SPSS software version 10.0, utilizing the Independent-Samples T-test to determine differences between tumor control and treated animals with a significance criterion of $p<0.05$.

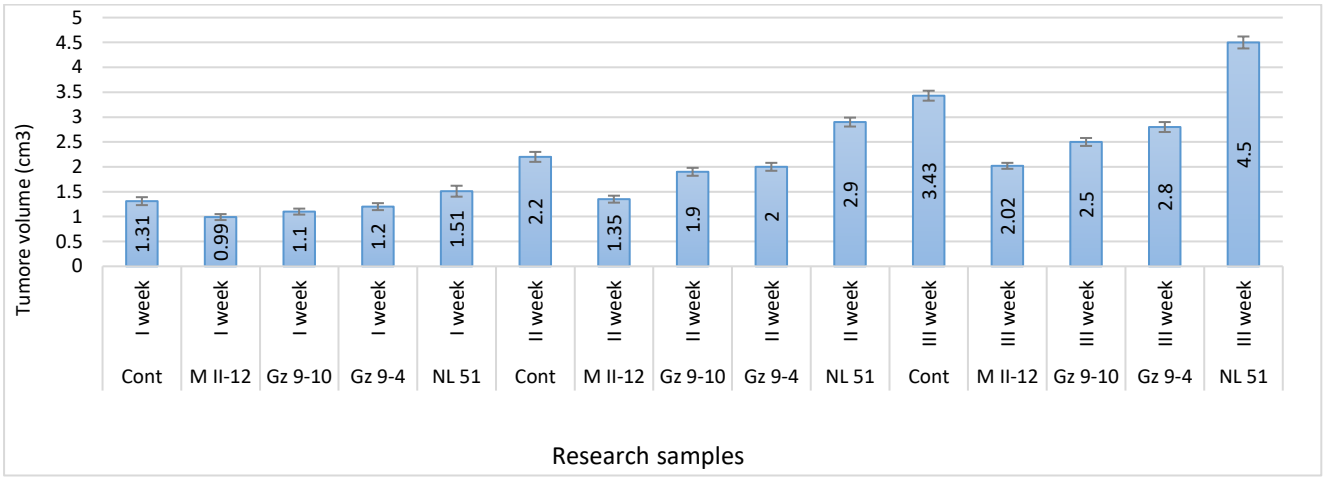
Results and discussion. Following the administration of test solutions in an in vivo Ehrlich carcinoma model, the lifespan of mice was monitored. The control group exhibited an average lifespan of 32 days. Conversely, in M II-12 treated group mice the lifespan was extended by 20 days ($p<0.001$), reaching 52 days compared to the control group. Injection of Gz 9-10 resulted in an 8-day ($p<0.05$) increase in mouse viability. Gz 9-4 samples had no significant effect on mice lifespan and NL 51 injections even decreased lifespan by 7 days ($p<0.05$) compared to control.

Fig 1. The lifespan of Ehrlich's carcinoma bearing mice treated with M II-12, Gz 9-10, Gz 9-4 and NL-51



Research samples were administered on the 7th day following the modeling of Ehrlich carcinoma in mice. Using a caliper the tumor tissue volume was measured. The results are shown on Fig. 2.

Fig 2. Tumor volume after influence of research samples: M II-12, NL-50, Gz-9-4, and Gz-9-10.



Initially, in the first week of experiment the control group animals' tumor tissue volume was 0.9 cm³. Administration of M II-12 effectively slowed down tumor growth compared to the control group, maintaining this effect consistently throughout the second and third weeks.

In case of Gz 9-10 test solutions, fewer fluctuations in tumor volume were noted compared to the M II-12 sample. The tumor volume was 1.1 cm³ in the first week, 1.9 cm³ in the second week, and 2.5 cm³ in the third week.

In the first week of experiment the tumor volume in Gz 9-4 -treated group was 1.2 cm³, in the second week - 2 cm³, and in the third week - 2.8 cm³. Conversely, an increase in tumor volume was observed in the NL51-treated group animals compared to the control.

Measuring Proteolytic and Inhibitory Activity Using Colorimetric Assay with EWMP Substrate.

Due to the significant role of proteolytic enzymes in tumor growth and development, investigations were conducted to evaluate their activity in the tested samples. Certain proteases can potentially promote tumor progression, the use of substances containing protease inhibitors presents a potential strategy for suppressing malignant growth. Thus, both proteolytic enzyme activity and protease inhibitor levels were determined using a colorimetric assay (see Table 1).

Table 1. Proteolytic activity of M II-12, NL-50, Gz-9-10 and Gz-9-10.

Name	Proteolytic activity A (U/ml)	Inhibitory activity A (U/ml)
NL-51	0.214	0
Gz 9-4	0.014	0
Gz 9-10	0.01	0
M II-12	0.112	0.064

As illustrated in the data provided, the samples NL-51, Gz 9-10, and M II-12 exhibited proteolytic activity. M II-12 additionally revealed inhibitory activity against proteolytic enzymes that support the cancer growth. Although, M II-12 exhibits the inhibitory property on proteolytic enzymes, it does not inhibit the proteolytic enzymes presented in tested M II-12 solution itself. This observation suggests the inhibitor's specificity toward certain proteases and potential synergistic action between these enzymes and inhibitors, which may contribute to the positive anticancer effect.

Conclusion. The obtained results indicate the potential therapeutic effects of the tested M II-12 sample. M II-12 significantly extended the lifespan of mice compared to the control group. This effect was also reflected on the tumor growth dynamics, where M II-12 exhibited a notable slowdown in tumor growth compared to the control group and other test samples. The Gz 9-10 sample showed a moderate increase in mouse viability. NL-51 had no significant inhibitory impact on tumor progression and even decreased lifespan of experimental animals.

The colorimetric assay results supported our findings by demonstrating proteolytic activity in all tested samples, and inhibitory activity of M II-12 on proteolytic enzymes supporting cancer growth. This specificity of certain proteases indicate a targeted mechanism of action, potentially contributing to its effectiveness in suppressing tumor growth.

Overall, our study highlights the potential of M II-12 as a promising candidate for further investigation as an anti-cancer agent. Further research focusing on elucidating the specific mechanisms underlying these effects could provide valuable insights for the development of novel therapeutic strategies against cancer.

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