ANTITUMOR ACTIVITY OF PLEUROTUS OSTREATUS GEMMOTHERAPIC EXTRACT

I. Pauliuc¹, Adinela Cimporescu², Cristina Vlad Daliborca²,³, Roxana Popescu⁴, Dorica Botau¹, V. Dumitrascu²,³

¹ FACULTY OF HORTICULTURE AND FORESTRY, UNIVERSITY OF AGRICULTURAL SCIENCES AND VETERINARY MEDICINE OF BANAT, TIMISOARA, ROMANIA
² SCJUT, TIMISOARA, ROMANIA
³ UNIVERSITY OF MEDICINE AND PHARMACY “VICTOR BABES” TIMISOARA, DEPARTAMENT OF BIOCHEMISTRY AND PHARMACOLOGY, TIMISOARA, ROMANIA
⁴ UNIVERSITY OF MEDICINE AND PHARMACY “VICTOR BABES” TIMISOARA, DEPARTAMENT OF CELLULAR AND MOLECULAR BIOLOGY, TIMISOARA, ROMANIA

Summary

Recently, the therapeutic potential of herbal biological compounds is intensively investigated in order to develop new pharmacological strategies with minimal systemic toxicity. Pleurotus ostreatus is a common edible mushroom which has shown promising anticancer activities in vitro and in vivo. The aim of our study was to investigate the anticarcinogenetic effects of a gemmotherapic extract of Pleurotus ostreatus. The extract was prepared from young parts of P. ostreatus, in accordance to the gemmotherapic principles. The cytotoxic effect of different concentrations of extracts was tested in HCT-116 cell line (colorectal carcinoma cell line) by MTT assay (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide). The result revealed that the extract had a significant inhibitory activity. The results indicate that the gemmotherapic extracts can be a viable alternative to the modern extraction techniques.

Key words: Pleurotus ostreatus, gemmotherapic extract, antitumor activity

ivan.pauliuc@gmail.com

Introduction

Gemmotherapy or plant stem cell therapy as it is known today uses a wide variety of embryonic plant parts, collected in the spring at a critical stage in the plants growth when much of the plants energy is directed to the growing areas. Gemmotherapy is an important subsection of phytotherapy. Gemmotherapic extracts are known for their higher content in active compounds (Rozencwajg, 2008). They are prepared according to gemmotherapic principles and consist in maceration of plant buds with equal thirds of water, alcohol and glycerin.

In recent years, there have been a revival of natural, plant based antimicrobial agents. This trend is the consequence of the limited effectiveness of synthetic products to fight against newer, drug resistant bacteria. For this purpose, the antimicrobial properties of many plant compounds from a wide variety of plant species have been assessed (Karuppusamy, 2009). Many species of macrofungi have long been used as food and as traditional medicines around the world since ancient times, especially in Asia. Some of this species exhibiting a haematological, antiviral, antitumour, antibiotic, antibacterial, and immunomodulating activities.

The genus Pleurotus from the family Pleurotaceae is a cosmopolitan group, including several cultivated species such as
P. pulmonarius, P. cornucopiae, P. sajorcaju, P. eryngii, P. cystidiosus, and P. ostreatus (Kuznetsov et al., 2005). To date approximately 70 species of Pleurotus have been recorded. Many of these species exhibit antimicrobial and antitumor properties (Akyuz and Kirbag, 2009; Maness et al., 2011). Fungi of the Pleurotus genus have an important place among the commercially basidiomycetes because they have gastronomic, nutritional and medicinal properties and can be easily cultivated on a large range of substrates.

In this study, we explored a new approach by testing a gemmotherapeutic extract from young parts of P. ostreatus based on the principle that gemmotherapeutic extracts have a more intense inhibitory activity compared with the traditional extracts (Rozencwajg, 2008).

**Materials and Methods**

**Plant material collection**

Strains of P. ostreatus were cultivated in a greenhouse at the University of Agricultural Sciences in Timisoara. Young parts were collected from very young mushrooms and put immediately in ethanol of 96% concentration.

**Preparation of gemmotherapeutic extracts**

The solutions were made with equal thirds of alcohol, glycerol and distilled water. The fresh buds were collected, cleaned, washed with distilled water and then put in the solution for extraction. The process of extraction took place for a week in a dark place at 10°C, using an orbital shaker. The extract was then filtered, concentrated and weighted. The dry material was diluted for the tests and filtered through a sterile membrane filter.

**Reagents**

DMEM (Dulbecco’s modified Eagle medium), fetal bovine serum, antibiotics were purchased from Lonza (Biozyme SA, Cluj-Napoca). MTT assay was purchased from Sigma – Aldrich.

**Cell culture**

HCT-116 cell line (colorectal carcinoma cell line) was cultured in DMEM with 10% fetal bovine serum and 1% penicillin/streptomycin. The cells was incubated at 37°C in humidified atmosphere of 5% CO2, 95% air.

**Treatment with P. ostreatus extracts and MTT colorimetric assay**

5 × 10^3 HCT-116 cells/ml was seeded in 96-well plates. After 24 h, cells were treated with different concentration (0.5mg/ml, 1mg/ml, 1.5mg/ml, 2mg/ml) of gemmotherapeutic extract. Untreated cells were used as controls. Microplates were incubated for 24h and 48h and the cytotoxicity was measured with colorimetric assay based on the use of tetrazolium salt MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide). The results were read on a multiwell scanning spectrophotometer at 570nm (Tecan Reader). The tests were performed in triplicate. The results are expressed as percent of cell proliferation inhibition calculated according to the following (Wu and Wang, 2010):

\[
\text{inhibition rate of tumour cells} \; (\%) = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100%.
\]

**Cell morphology**

was analyzed using Nikon Eclipse Inverted Microscope.

**Statistical analysis**

Data were averages of three results ± Standard Deviations (SD) by using Microsoft Excel.

**Results and discussions**

The inhibition of proliferation in HCT-116 cancer cell line was reduced at 24 h, and significant at 48h (fig. 3,4,5).
Fig. 1. Untreated control after 24h

Fig. 2. Cell treated with 2mg/ml extract, after 24h

Fig. 3. Untreated control after 48h

Fig. 4. Cell treated with 0,5mg/ml extract, after 48h

Fig. 5. Cell treated with 2mg/ml extract, after 48h

Fig. 6. Inhibition of cells proliferation at 24h and 48h

of extracts (0,5mg/ml and 1mg/ml) and At 24h after exposure, the elevated concentration of gemmotherapic extract of *P. ostreatus* (1,5mg/ml and 2mg/ml) induced an inhibition of cell proliferation compared with low concentration untreated control. After 48h, and the effect increased in a dose-dependent manner (fig.6).

The results obtained, provide experimental evidence that gemmotherapic extracts of the mushrooms *P. ostreatus* are potential sources of anticancer compounds. The mushroom extracts can also be used in combination with traditional chemotherapy. Also, mushroom extracts might be considered alternative sources for adjuvant cancer therapy, as they have no adverse effects and they activate the cells of the immune system (Barros et al., 2007). The bioactive compounds of mushrooms
complement classical cancer therapy, and countering the side-effects of cancer, such as nausea, bone marrow suppression, anemia, and lowered resistance (Patel and Goyal, 2012).

**Conclusions**

Based on the results obtained from the present study, it can be concluded that the gemmotherapeutic principles can be successfully applied for mushroom extracts in the development of more potent and efficient antitumor agents. Also, the gemmotherapeutic extracts obtained from *P. ostreatus* young parts, using the classic gemmotherapeutic principles, exhibit an antitumor activity.

**References**


