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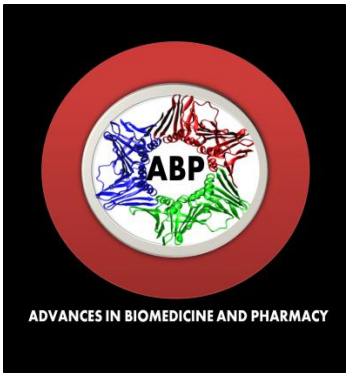

Original Article

Anticancer potential *of Trametes versicolor* (L.) Lloyd and *Auriporia aurea* (Peck) Ryvarden mycelia in rat Guerin's carcinoma.

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Abstract

Mushroom extracts possess promising immunomodulatory and anticancer potential which develops interest for new entities from these fungal sources for novel therapeutic agents against different diseases. In present study Anticancer activity of aqueous extracts of two fungal species, *Auriporia aurea* 5048 and *Trametes versicolor* 353 mycelia cultivated on a natural substrate (amaranth flour – waste of CO_2–extraction from *Amaranthus hybridus* L. grains) have been investigated for their anti-cancer potential using Guerin's carcinoma as a tumor model. The effectiveness was evaluated by the degree of tumor growth inhibition based on tumor volume. The administration of mushroom suspensions and extracts resulted in significant tumor size decrease compared to the control group (from 7th to 16th day after Guerin's carcinoma inoculation). It has been found that that oral dose of *T. versicolor* mycelium aqueous extract two times a day for 10 consecutive days inhibited tumor growth by 65% and prolonged administration of *T. versicolor* mycelium suspension was accompanied by tumor size growth at the terminal period (22th day), which twice exceeded the performance of the control group. Further, to the best of our knowledge the anticancer potential of *A. aurea* against carcinoma was reported for the first time in this study.

Keywords: *Trametes versicolor*, *Auriporia aurea*, mycelium aqueous suspension, mycelium aqueous extract, Guerin's carcinoma.

Introduction

Cancer is one of the leading causes of mortality worldwide. According to the reports, cancer-related deaths will increase to over 24 million in 2035 [1]. Prevention and treatment of cancer remain an imperative problem in modern medicine. To date, experimental and clinical observations proved the promising use of natural products, with no significant side effects. They may have a dual mechanism of action: directly prevent carcinogenesis and metastasis and promote the involvement of immune response mechanisms, since it is known that tumor growth is accompanied by significant alterations in immune system. Higher mushrooms, in particular *Basidiomycetes*, contain a powerful complex of biologically active compounds, including polysaccharides. Two directions of mushroom application are under research development: expansion of the list of mushroom species with immunomodulating and anticancer properties and in-depth study of all aspects of specific mushroom with regard to biologically active compounds and their application in oncology. It should be noted that the anticancer activity is

not only restricted to mushroom species but it is strain specific also. The best known commercial polysaccharopeptide preparations from fungi are PSK (Polysaccharide-K) and PSP (Polysaccharide peptide). Both products are obtained by extraction of *Coriolus versicolor* (*Trametes versicolor*) mycelia and have similar physiological activities, however they are structurally different. PSK is produced from CM-101 strain of *C. versicolor* by ammonium sulfate extraction from hot water extract. PSP is produced from Cov-1 strain of *C. versicolor* by alcoholic precipitation extraction from hot water extract [2]. It has been reported [3] that antitumor activity of Basidiomycetes preparations is correlated with antiviral activity. Taking into account high antiviral activity [4] of *T. versicolor* (strain 353) and little-studied fungus *Auriporia aurea* (strain 5048) the investigation of anticancer activity of both mushrooms is of imperative interest. Another important question was the method adopted for mushroom preparations processing. The most common forms of mushroom preparations are extracts [2] and powders (suspensions) [5]. However the comparative studies regarding the effectiveness of certain substances (fractions isolated from the fungus) or mushroom powder (complex of biologically active substances) application are not well presented in the literature.

The aim of the present study was to assess the antitumor activity of aqueous extracts and aqueous suspension of *A. aurea* and *T. versicolor* mycelia cultivated on a natural substrate (amaranth flour – waste of CO_2– extraction from *Amaranthus hybridus* L. grains), using Guerin's carcinoma as a tumor model.

Materials and Methods

Fungi species and Growth Conditions

The fungi species *Auriporia aurea* 5048 (Peck) Ryvarden and *Trametes versicolor* 353 (L.) Lloyd. were kindly supplied by the Culture Collection of Mushrooms (IBK) of the M.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine [6]. Mycelial cultures were initially grown in Petri dishes (90 mm diameter) on culture medium with pH 6.0, composed of (g/L): 20.0 glucose, 3.0 yeast extract, 2.0 peptone, 1.0 K_2HPO_4, 1.0 KH_2PO_4, 0.25 $MgSO_4$ x $7H_2O$, and 20.0 agar. The liquid culture medium (substrate – 60 g amaranth flour in 1 L distilled water) was sterilized by autoclaving for 20 min at 121ºC. Flasks (250 ml) with 50 ml liquid medium were inoculated with three mycelial plugs of 8 mm diameter cut from the Petri dishes using a sterile borer at the stage of actively growing mycelia. Mycelia were grown as static cultures in flasks for 14 days at 26 ± 2ºC. The mycelium was separated from the medium by filtration through Whatman's filter paper № 4 and washed with distilled water, dried (Desiccator SNOL-3,5.3,5.3,5/3,5-I1M, Smolensk, Russia) to constant weight at 50±2°C and ground to power consistent using a blade grinder.

The preparation of experimental drugs

Aqueous suspensions and extracts of above-mentioned fungi mycelium were tested using Guerin's carcinoma as a tumor model. The suspension was obtained by suspending 2 g of fungi mycelium in 10 ml of distillated water. The aqueous extracts of fungi were prepared as follows: a sample (2 g of each dry mycelium in 10 ml distillated water) was extracted for 4 h in a water bath at 60 °C with periodic stirring for 24 h, after extraction insoluble compounds were separated by centrifugation at 6.000 x g for 10 min and the supernatant was used in the study.

Experimental animals and tumor transplantation

White outbred rats (n=34) aged 2.5–3 months and mass of 90–100 g were housed on a standard diet of vivarium in plastic cages (three per cage) with sand substrate. Guerin's carcinoma (GC − adenocarcinoma of rat uterus) was used as tumor model. GC was transplanted subcutaneously to the hip by standard method (0.4 ml of a 30% suspension of tumor cells in saline). GC strain of the tumor was kindly supplied by the RE Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, National Academy of Sciences of Ukraine (Kyiv, Ukraine). After 6 days of GC-cell inoculation the animals were subdivided into the following groups:

group I – (P, control) – 10 animals receiving 1 ml of saline solution (intragastric *per os*) every 12 hours;

group II – (P+E1) – 6 animals receiving 1 ml of aqueous extract from *T. versicolor* mycelium (intragastric *per os*) every 12 hours;

group III – (P+S1) – 6 animals receiving 1 ml of aqueous suspension from *T. versicolor* mycelium (intragastric *per os*) every 12 hours;

group IV – (P+E2) − 6 animals receiving 1 ml of aqueous extract from *A. aurea* mycelium (intragastric *per os*) every 12 hours;

group V – (P+S2) – 6 animals receiving 1 ml of aqueous suspension from *A. aurea* mycelium (intragastric *per os*) every 12 hours.

The extracts and suspensions were administered on a daily basis during all the experimental period (until the death of

the animal). Primary tumor volume and lifetime were monitored daily.

All the procedures were performed in accordance with principles of the European legislation [7] and standards of biomedical ethics of Ukraine [8].

The effectiveness of anticancer drug action was evaluated by the degree of tumor growth inhibition based on tumor volume calculated according to the ellipsoid volume formula: $V = \pi/6 \times (L \times V \times H)$, where: *L* – length, *W* – width, *H*– height [9].

The tumor growth inhibition rate (IR) was determined by the formula: IR= [(tumor volume of control group – tumor volume of experimental group)/tumor volume of control group] × 100% [10]. Tumor growth inhibition >50% is considered meaningful.

Statistical Analysis

All the results were statistically treated using the Student's *t*- test. Differences at $p \leq 0.05$ were considered to be significant.

Results

The present study was performed to evaluate anticancer activity of aqueous suspensions and extracts of *T. versicolor* and *A. aurea* mycelia. The effects of the studied preparations were examined after oral administration.

The administration of aqueous extracts and suspensions of mycelia resulted in decreased tumor volume compared to the control group, but only during the period of active growth of the tumor (7th to 16th day after inoculation of Guerin's carcinoma) (Fig. 1A). In the studied period the growth of malignant neoplasms (day 16), the volume of tumors in all treatment groups were lower compared to the control. Tumor volumes of animals receiving *T. versicolor* mycelium suspension, *A. aurea* mycelium extract and suspension were less compared to control group, at 36 %, 27 % and 20 % respectively (Fig. 2 A), whereas tumor growth inhibition degrees were 38.5 %, 29.9 %, and 20.5 %.

The maximum difference between the indices of tumor volume of animals in the experimental and control groups was recorded in the case of application of *T. versicolor* mycelium aqueous extract. From the Figures 1(A), 2(A), it is clear that the measures of the tumor volume in animals of this group 16 days after transplantation of GC (the period of active growth of the tumor) were lower by 65 % when compared to the control group of animals (tumor growth inhibition degree was 65.1 %). Thus an intake of *T. versicolor* mycelium aqueous extract two times a day for the first 10 consecutive days demonstrated a significant tumor growth inhibition.

While investigating the anticancer activity of a drug, life expectancy is considered to be an important factor.

Note (here and later): P (control) – animals with transplanted GC (n=10); P+E1 – animals with transplanted GC treated with the aqueous extract of *T. versicolor* mycelium (n=6); P+S1 – animals with transplanted GC, treated with a suspension of *T. versicolor* mycelium (n=6); P+E2 – animals with transplanted GC treated with the with aqueous extract of *A. aurea* mycelium (n=6); P+S2 – animals with transplanted GC, treated with a suspension of *A. aurea* mycelium (n=6).

It has been observed that the administration of *T. versicolor* mycelium aqueous extract was characterized by the lowest levels of lethality among the animals of the experimental groups throughout experimental period. At the same time, long-term application of a suspension of mycelium of this fungus (group P+S1), was accompanied by tumor growth at the terminal period of growth (22nd day), which was two times higher than the rates in the control group (Fig. 2B). Administration of *A. aurea* mycelium aqueous extract (group P+E2) and suspension (group P+S2) led to the premature death of the animals in these groups. So the current study indicates that animal mortality rate of these groups on the 19th day of the experiment was 33 % and 50 %, respectively, as well as 100% – on the 22nd day.

The analysis of lifespan indices of animals with GC revealed an increase in life expectancy to 20 % (after 21 days of experiment) in animals receiving *T. versicolor* mycelium aqueous extract. These animals continued to live even at 35 days after GC transplantation.

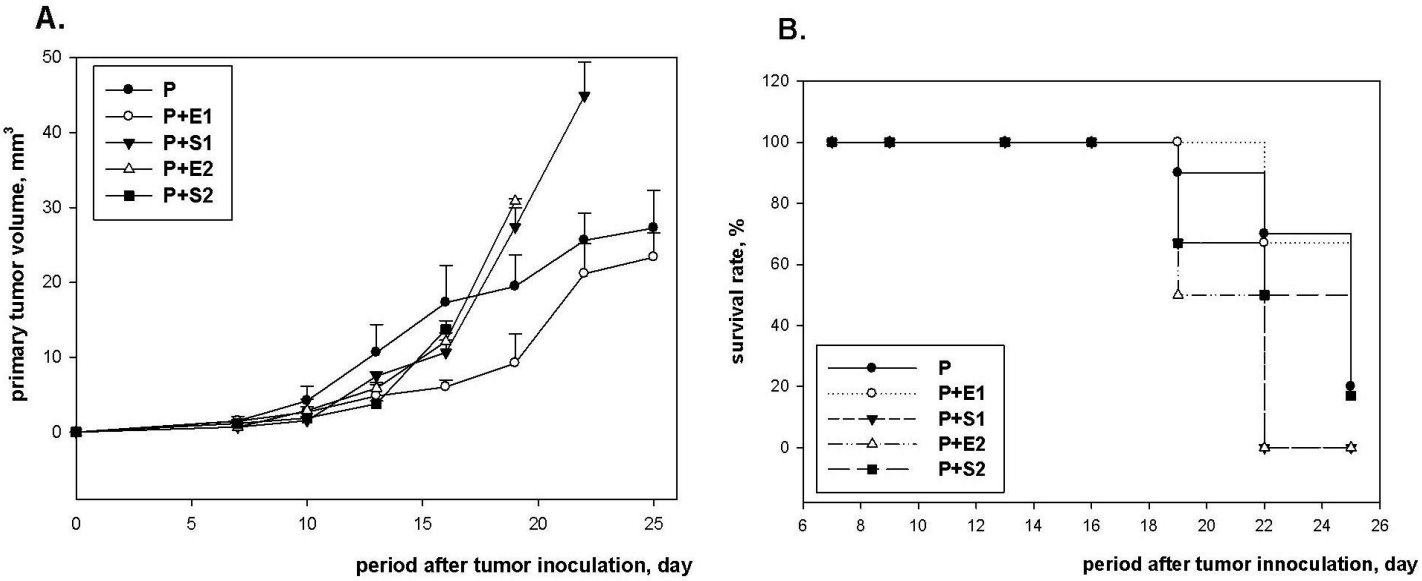

Figure 1: Primary tumor volume of GC (A) and the survival of tumor-bearing animals (B).

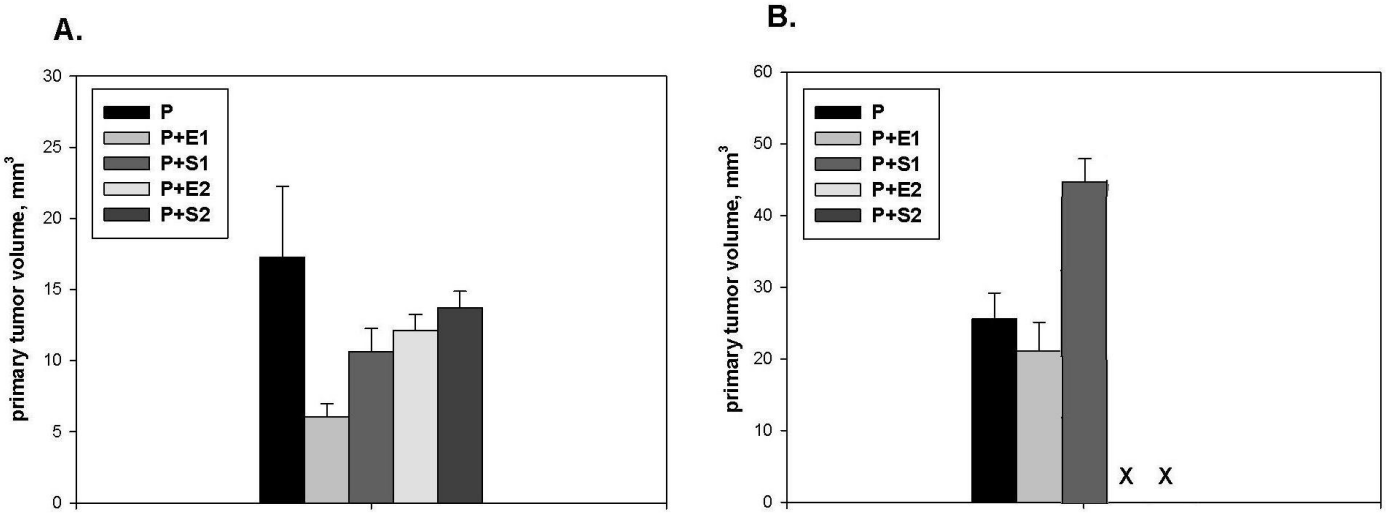

Figure 2: Primary tumor volume in animals in the period of GC active growth (A, day16) and at the terminal stages of tumor growth (B, day 22). Note: X – no indicators in this group due to 100 % mortality in this period of the experiment.

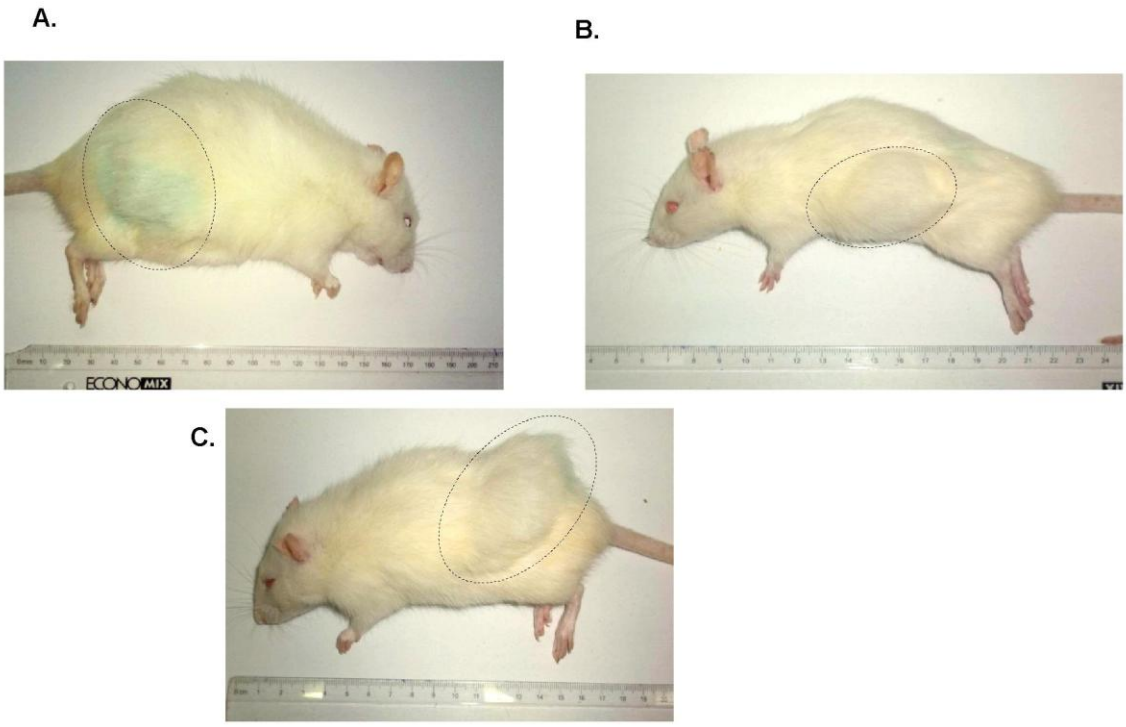

Figure 3: Representative photographs of primary tumors in rats in the control group (A, group P) and groups treated with aqueous extract of the mycelium of *T. versicolor* (B, group P+E1) and a suspension of mycelium of *A. aurea* (C, group P+S2) for 22 days after transplantation of GC.

Discussion

The present study has revealed anticancer potential of *Trametes versicolor* 353 (L.) Lloyd. and *Auriporia aurea* 5048 (Peck) Ryvarden mycelia (aqueous suspensions and extracts) against GC.

Ideally, it is desirable to compare the results of anticancer activity (the same cancer model) of one type of fungi and the same strain because there is a difference in the activity of different strains [2]. We selected GC model for our study as Kukharchuk et al. [11] have confirmed the possibility of GC model using in real conditions for studying the effectiveness of definite antitumor preparations.

The method of making a preparation and the method of its administration has a great significance.

The reference for comparison of anticancer activity of *A. aurea* does not exist since it has not been studied previously. *T. versicolor* anticancer activity was investigated in many studies and reported in many reviews (in experimental cancer models and clinical trials) [2, 5, 12–15]. The majority of reviews and studies are related to the activity of the extracts from this fungus [12, 16]. The preparation of these extracts are aimed primarily at extracting polysaccharides and polysaccharide-protein complexes. Most of the clinical trials are related to the integrated cancer therapy, including the reception of fungal drugs to compensate the side effects of two main methods of treatment – chemotherapy and radiotherapy, showing safety of mushroom products and improving life quality of the patient.

Besides the suspension and extract preparation method used in the current study, it is imperative to concentrate on the mode of administration. Based on the previous literature we have employed oral route in our study. It has been found that some routes of administration although resulted in positive results in case of fungal preparations but at the same time it was .accompanied with side effects [15] and even progression of the disease [17- 19].

Systematic review and meta-analysis technique were used by Wong et al. [15] to aggregate and analyze the efficacy of *T. versicolor* on survival in cancer patients from 13 clinical trials. The findings have shown that *T. versicolor* administration results in a significant survival advantage compared with standard conventional anti-cancer treatment alone. Of patient randomized to *T. versicolor*, there was a

9% absolute reduction in 5-year mortality, resulting in one additional patient alive for every 11 patients treated. In patients with breast cancer, gastric cancer, or colorectal cancer treated with chemotherapy, the effects of the combination of *T. versicolor* preparation on the overall 5-year survival rate was more evident, but not in esophageal cancer and nasophayngeal carcinoma. However, subgroup analysis could not conclude which type of anti-cancer treatment may maximize the benefit from *T. versicolor*. *Use of T. versicolor* has been associated with certain side effects (leucopenia, elevated LDH, elevated GOT, elevated GPT, anorexia, nausea/vomiting, diarrhea) in comparison with the control, especially in patients with breast cancer. These findings highlight the need for further evidence from prospective studies of outcome to guide future potential modifications of treatment regimes.

The results of Avtonomova *et al.* showed that the biomass aqueous extracts of crude *C. sinensis* and *C. sobolifera* submerged biomass had a strong incentive effect on the growth of subcutaneous tumor nodes in the used model. The tumor grew two times faster in mice treated with aqueous extracts of these fungi than in controls. The aqueous extract of raw *C. ophyoglosoides* biomass had weak inhibitory effect on tumor growth. Preparation combining ethanol and water extracts of dry *C. sobolifera* biomass, showed a stronger antitumor effect than the dry submerged biomass of this fungus [19].

Cui and Chisti [14] discussed properties, physiological activity, recovery, and purification of *T. versicolor* bioactive polysaccharopeptides

Taking into account the data obtained by other researchers and given above it can be assumed that the suspension of the fungi contain substances that accelerate the development of cancer, particularly in the terminal period of the disease.

One of the important steps of investigations is understanding and interpretation of mechanisms of action of antitumor activity. As the mechanisms of antitumor activity still remain largely unknown. A number of researchers [2, 13, 20, 21], in the study of polysaccharides and polysaccharide-protein complexes isolated from *T. versicolor*, assumes a dual mechanism of action: the possible direct cytotoxic effect (inhibition of the uncontrolled growth of cancer cells) and "inclusion" of immune mechanisms of the body.

Conclusion

The present study revealed anticancer activity of *Trametes versicolor* 353 (L.) Lloyd. and *Auriporia aurea* 5048 (Peck) Ryvarden mycelia (aqueous suspensions and extracts) against GC.

It was found that mycelia aqueous extracts of the studied species have greater activity compared to their aqueous suspension. The oral administration of suspensions and extracts to rats resulted in tumor size decrease in all treatment groups compared to the control group, but only in the period of active growth of the tumor (from 7th to 16th day after inoculation of GC). An intake of *T. versicolor* mycelium aqueous extract two times a day for 10 consecutive days demonstrated a significant tumor growth inhibition, but do not affect the rate of growth of tumors in terminal period. One of the most important results of the study have revealed that prolonged administration of *T. versicolor* mycelium aqueous extract leads to the tumor growth almost to the size of the control, suspension of *T. versicolor* mycelium causes increased tumor growth (2 times larger than control), aqueous extract of *A. aurea* and suspension of its mycelium accelerated the death of experimental animals. The cause of this may be the effect that preparation from the same fungus may have the opposite effect at different stages of cancer. Subsequent research must be devoted towards the determination of the qualitative and quantitative composition of *T. versicolor* mycelium and its aqueous extract, isolation of biologically active compounds with antitumor activity and elucidation of the precise mechanisms of this activity.

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None declared

Conflict of interest

The authors declare that there is no conflict of interest to reveal.

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