Effect of Medicinal Mushroom, *Auricularia auricula-judae*, polysaccharides against EAC cell lines

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Abstract

One of the major causes of mortality worldwide is cancer. Increasing research on herbal medicine has revealed its importance in treating many diseases including cancer. The present study was carried out to evaluate the antitumor activity of crude polysaccharide extract of *Auricularia auricula-judae* on Ehrlich Ascites Carcinoma (EAC) model in mice. After inoculation of EAC cells into mice, treatment with *Auricularia auricula-judae* (AAE) (200 mg/kg) was continued for 9 days. The effect of drug response was evaluated by the study of tumor growth response including study of hematological parameters, biochemical analysis, chromosomal disintegration assay and in vitro cytotoxicity. Experimental results revealed that the polysaccharide extract of *Auricularia auricula-judae* possesses significant antitumor activity due to the presence of polysaccharides like Beta-Glucans which may be in response to its cytotoxicity.

Keywords: *Auricularia auricula-judae*, AAE, EAC cells, Beta-Glucans, Cytotoxicity.

Introduction

Over the past few decades, cancer has remained as the largest cause of mortality worldwide and the number of individuals living with cancer is steadily expanding. Hence, a major portion of the current pharmacological research is involved with the anticancer drug design customized to fit new molecular targets. Due to the enormous propensity of plants, which synthesize a variety of structurally diverse bioactive compounds, the plant kingdom is a potential source of chemical constituents with antitumor and cytotoxic activities. Traditionally various plants have long been used in the treatment of cancer.

*Auricularia auricula-judae* is a common jelly fungus widely distributed throughout North America and Europe. It is brown, irregular, wavy, rubbery and gelatinous; it grows on wood; it is edible but with no distinctive taste and is widely cultivated throughout the world for use as mushroom as well as medicine. The mushroom has been used traditionally as medicine in many countries as anti-diabetic, antitumor, antihypertensive, anti-inflammatory, immunomodulatory and antibacterial agents. Several *in vitro* and *in vivo* studies with crude mushroom extract as well as various purified fractions, including proteins and polysaccharides, have shown antitumor activity and it is found to be Beta-glucan which is in the cell walls of mushrooms where it is firmly bound to other molecules responsible for the strength and shape stability of the cells. Besides beta-glucan, the cell wall also contains other saccharides and proteins that are linked and bound together to form a solid structure which makes it responsible for anticancer activity. However, in spite of traditional use, pharmacology of its parts has not yet been explored scientifically. The present investigation was carried out to evaluate the anticancer property of *Auricularia auricula-judae* against Ehrlich Ascites Carcinoma (EAC) tumor model.

Material and Methods

Biochemical screening: The preliminary biochemical screening was carried out by estimating the moisture content, carbohydrate, nitrogen, crude protein, amino nitrogen, fat, magnesium, phosphorus, crude fibres and ash content of the dried powder of *Auricularia auricula-judae* and the percentage of each molecule are tabulated in table 1.

Extraction of polysaccharides from *Auricularia auricula-judae*: The anti-cancer compounds are extracted from mushroom fruit-bodies. In the initial step dried mushroom powder was repeatedly heated in 80% ethanol to extract and eliminate low molecular weight substances. Crude fractions 1, 11 and 111 are obtained from the remaining ethanol extract residue by extraction with water (100°C, 3h), 1% ammonium oxalate (100°C, 6h) and 5% sodium hydroxide (80°C, 6h). The obtained fractions were evaporated, dried and purified. The prepared extracts were used for the *in vitro* and *in vivo* studies of anticancer activity.

Purification of polysaccharides: Column chromatography was performed with DEAE-cellulose. Volumes of 10 ml of the extracts (different polysaccharides obtained) at 400 mg/L were applied. The column was eluted with deionized water, NaCl at different concentrations (0.05, 0.10 and 0.50 M), NaHCO$_3$ (0.3 M) and NaOH (0.1 M) successively at the flow rate of 30 ml/h. The isolated fractions were measured by the phenol-sulphuric acid method. Finally, different fractions of isolated polysaccharides were obtained and lyophilized for 48 h for other assays. Since the yields of the purified fractions were very low, crude polysaccharides extract were prepared for *in vivo* studies.

Anti-Cancer Screening

Cell line: Cancer cell lines, Ehrlich ascites carcinoma (EAC), to induce cancer in animal model (mice) were...
obtained from Amala Cancer Research Center, Amala Nagar, Kerala, India. The cells were maintained as ascites tumor in Swiss Albino mice by intraperitoneal injection of 1×10⁶ viable cells.

Animals: Swiss albino mice (25-30 g) were procured from KMCH, Coimbatore, Tamilnadu, India and used throughout the study. They were housed in microlon boxes in a controlled environment (temperature 25±2°C and 12 h dark/light cycle) with standard laboratory diet, water at libitum approved by the Institutional Animal Ethical Committee (IAEC).

Experimental Protocol: Healthy Swiss albino male mice were weighed and divided into three groups (n=6). EAC cells (1 × 10⁶ cells/ mouse) where injected i.p. to each mouse of each group except normal saline group. This was taken as day 0. Extract treatment was continued for subsequent 9 days starting from day 1. On 10th day, 24h after the last dose, mice were sacrificed from each group. Before that the animal blood was collected to evaluate the hematological and biochemical parameters. The groups and the design of the experiment were as follows:¹⁴

Group I: Normal Saline Group
Group II: Tumor control - EAC (1×10⁶ cells / mouse, i.p.)
Group III: Treated -EAC (1x10⁶ cells / mouse, i.p.) + AAE (200mg/kg b.wt, p.o.)

Antitumor activity of AAE was assessed by observation of changes with respect to the following parameters:

Tumor cell count: The ascetic fluid was taken in a WBC pipette and diluted 100 times. Then a drop of the diluted cell suspension was placed on the Neubauer counting chamber and the numbers of cells in the 64 small squares were counted [Table 2].

Cytotoxicity Assay: In vitro short term cytotoxic activity¹⁸ of Auricularia auricula-judae was determined using EAC cells directly from the control and test mice intraperitoneally.100µl of 0.4% Trypan blue dye solution was added to both sample and mixed well. After mixing, the live and dead cells were counted in haemocytometer and the percentage of cytotoxicity was calculated.

% of Cytotoxicity = No. of dead cells / No. of live cells + No. of dead cells X 100.

Haematological Study: In order to detect the influence of mushroom on hematological status of EAC bearing mice, a comparison was made among three groups (n=6) of mice on the 14th day after inoculation. The groups comprised of (1) Tumor bearing mice (2) Tumor bearing mice treated with AE (200 mg/Kg/day, p.o. for 9 days) (3) Control mice (Normal Saline group). Blood was drawn from each mice by the retro orbital plexus method and the White Blood Cell count (WBC), Red Blood Cells (RBC) hemoglobin, protein and Packed Cell Volume (PCV) were determined.

Disintegration assay of Chromatin by Toluidene blue: The smear of culture was air dried and it was fixed in the mixture of ethanol: acetic acid (3:1) for half an hour. The slide was stained using 0.2mg/ml toluidene blue for 30 seconds and washed using distilled water and the disintegrated chromatin observed under light microscope (40X).

Results and Discussion

In the current study of tumor growth response, AAE treatment significantly reduced packed cell volume and viable cell count compared to those of EAC control mice, along with increase in nonviable cell count in the treated groups.

Haemotological parameters of tumor bearing mice on the day 14 showed significant changes when compared to normal mice. The total WBC count, protein and PCV were found to increase with a reduction in the haemoglobin content of RBC. The differential count of WBC showed that the percentage of neutrophils increased while that of lymphocytes decreased. Treatment with AAE brought back the haemoglobin content, RBC and WBC counts to the near normal.

Nuclear staining studies of the Cancer cells revealed the damages made by the extract on cancer cells. Cytotoxicity assay and the disintegrated chromatin were observed in the ascetic fluid and the percentage of disintegration was nearly same in all the three categories. The percentage of cell impact was found to be 33% through cell counting. The anticancer activity was also tested by culturing the treated and tumor control samples in MEME medium from the doubling time of cells.

Khan et al² studied the anticancer activity against EAC cells by examining the survival time of mice and the cytotoxicity test. In the present study performed cytotoxicity test and chromatin disintegration methods before and after the treatment in mice model was employed to identify the live and dead cells.

Tetsuro Ikekawa et al¹⁹ reported that the aqueous extract of seven edible mushrooms including Auricularis exhibited 33% inhibition against the EAC cell lines.

Conclusion

EAC has a resemblance with human tumors which are most sensitive to chemotherapy due to the fact that it is undifferentiated and it has a rapid growth rate. Due to this resemblance, EAC cell line can be used as a model for
human cancer. Tetsuro Ikekawa et al reported that 200 mg/Kg body weight of crude Auricularis extract showed anticancer activity against Sarcoma 180. The present study revealed that the polysaccharide extract of Auricularia auricula-judae is effective against EAC induced Swiss albino model with the dose of 200 mg / Kg showing significant anticancer activity and we also conclude that the polysaccharides play a major role in producing the anticancer activity.

Acknowledgement

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Table 1

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Components</th>
<th>% present in Auricularia auricula-judae</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Moisture</td>
<td>12.8</td>
</tr>
<tr>
<td>2</td>
<td>Carbohydrate</td>
<td>31.3</td>
</tr>
<tr>
<td>3</td>
<td>Nitrogen</td>
<td>1.5</td>
</tr>
<tr>
<td>4</td>
<td>Crude protein</td>
<td>6.5</td>
</tr>
<tr>
<td>5</td>
<td>Amino nitrogen</td>
<td>3.4</td>
</tr>
<tr>
<td>6</td>
<td>Fat</td>
<td>1.2</td>
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<tr>
<td>7</td>
<td>Magnesium</td>
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<td>8</td>
<td>Phosphorus</td>
<td>0.2</td>
</tr>
<tr>
<td>9</td>
<td>Crude fibre</td>
<td>6.7</td>
</tr>
<tr>
<td>10</td>
<td>Ash</td>
<td>5.4</td>
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</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Groups</th>
<th>EAC cell line</th>
<th>AE Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I (Normal)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>II (Tumor Control)</td>
<td>10⁶ cells mouse¹</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>III (Test)</td>
<td>10⁶ cells mouse¹</td>
<td>200 mg/Kg for 9 days</td>
</tr>
</tbody>
</table>

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