CHAPTER 6
ANTITUMOUR ACTIVITY OF THE METHANOLIC
EXTRACTS OF PLEUROTUS FLORIDA AND
PLEUROTUS SAJOR-CAJU
6.1 INTRODUCTION

Cancer is recognized as a complex, multifactorial disease caused, in part, by endogenous metabolic or other imbalances associated with age or genetic make up and, in part, by a wide variety of exogenous factors including diet, lifestyle, and exposure to ionizing radiation and chemicals of natural or man-made origin. Major advances in the treatment of cancer have emerged from the recent revolution in clinical interventions. However, significant heterogeneity in the efficacy and toxicity of chemotherapeutic agents is consistently observed across the human population (Evans and Relling, 1999). Administration of the same dose of a given anticancer drug to a population of patients results in a range of toxicity, from unaffected to lethal events (Sargent et al., 2001, Rothenberg et al., 2001). While many clinical variables have been associated with drug response (age, gender, diet, organ function, tumour biology), genetic differences in drug disposition and drug targets can have a great impact on treatment outcome (Evans and Johnson, 2001, McLeod et al., 2001). The general frustration by the public on the lack of effective strategies for both the prevention and treatment of cancer has become increasingly apparent. Treatment for cancer is frequently an assault to the immune system. The side effects of conventional anti-cancer modalities, whether through the process of radiation or chemical treatment, is a general weakening of the body's immune system resulting in immunosuppression that can significantly increase a patient's risk for infection. In such patient population, infection can quickly progress to sepsis, septic shock, and death. Pneumonia and sepsis are common
complications from chemotherapy and they are often lethal secondary effects that the modern oncologist, must face when ascribing treatment regimens. The targets of any anti-cancer treatment are the rapidly dividing cells within a tumour mass. The strategies used today try to selectively eradicate these rapidly dividing 'rogue' cells without adversely affecting other organs and tissues of the body. However, drug cocktails and radiation exposures are toxic to living cells. As a consequence, other proliferating cells also become targets of therapy regimes due to the toxic nature of treatment. These include follicular hair cells and the immune cells, which is manifested by hair loss and compromise of the body's general defense mechanisms. (Buchdunger et al., 1996, Kurzrock et al., 2003, Kidd, 2000).

Mushroom extracts are widely distributed as nutritional supplements and heralded as beneficial for health. The complementary and alternative medicine professions have disseminated much of the current awareness regarding the utility of mushrooms. Mushroom and plant polysaccharides are undergoing scientific analyses and development to prevent and treat cancer. It has become more and more apparent, especially in the Western World. The mushrooms characteristically contain many different metabolites with diverse medicinal properties (Gunde-Cimerman, 1999). Attempts have been made in many parts of the world to use several mushrooms and their metabolites for the treatment of large number of diseases. A number of anticancer drugs have been developed from mushrooms and some of these are extensively used in some parts of the world. Oyster mushrooms (Pleurotus
species) are found to have many medicinal properties. They have been reported to possess hypoglycemic, antithrombotic, hypotensive, hypolipidemic, and immunomodulatory activities (Chang, 1996). Investigations were carried out to evaluate the antitumour activity of extracts of \textit{P. florida} and \textit{P. sajor-caju} and findings are reported in this chapter.

6.2 MATERIALS AND METHODS

6.2.1 ANIMALS

Male Swiss albino mice of 4-5 week old and 20-25gm weight were used for the studies.

6.2.2 CELL LINES

Ehrlich’s ascites carcinoma (EAC) and Dalton’s lymphoma ascites (DLA) cell lines

6.2.3 PREPARATION OF THE EXTRACT

Methanolic extracts of both \textit{P. florida} and \textit{P. sajor-caju} were prepared as described in the section 2.2.1

6.2.4 ASSAY FOR CYTOTOXICITY

The in vitro cytotoxicity of methanolic extract of \textit{P. florida} and \textit{P. sajor-caju} were assayed using DLA and EAC cell lines. Briefly 1X10^6 viable cells of DLA or EAC cell line were suspended in 0.1ml of phosphate buffered saline (PBS) (0.2M, pH 7.4) various concentrations of extract (10\mu g/ml to 1mg/ml) and phosphate buffer in final volume of 1ml were incubated at 37°C for 3h. After the incubation the viability of the cells was determined by trypan blue exclusion method (Talwar, 1974).
6.2.5 ANTITUMOUR ACTIVITY

Antitumour activity of the extracts was determined using ascites and solid tumour models.

6.2.5.1. ASCITES TUMOUR MODEL

(a) EFFECT OF P. FLORIDA AGAINST ASCITES TUMOUR MODEL

The animals were divided into 4 groups with 6 animals in each group. The animals were injected intraperitoneally with 1X10^6 EAC cells in PBS (aspirated from 15 day old ascites tumour bearing mice). Methanolic extract of P.florida at doses of 500 and 1000 mg/Kg body weight were administered i.p 24h after the tumour implantation to two groups. The administration of the drug was continued for ten days with one doze in each alternate day and the total number of dose given was five (Jones and Janardhanan, 2000). The group administered with EAC cells alone was taken as positive control. Cisplatin (2mg/Kg body weight, i.p) was used as the standard reference drug. The mortality rates was noted in each group and the percent increase in life span (ILS) of the drug treated group was calculated using the formula \( \% \text{ ILS} = \left(1 - \frac{T}{C}\right) \times 100 \) (T is mean survival time of extract treated group and C- that of the control) (Ahluwalia et al., 1984).

(b) EFFECT OF P. SAJOR-CAJU AGAINST ASCITES TUMOUR MODEL

To study the effect of methanolic extract of P.sajor-caju animals were divided into 4 groups of 6 animals each group and the experiment was carried out as described above using methanolic extract of P.sajor-caju. The mortality rates was noted in each group and the percent increase in life span (ILS) of the drug treated group was calculated using the formula \( \% \text{ ILS} = \left(\frac{T}{C}-1\right) \times 100 \) (T is
mean survival time of extract treated group and C is that of the control) (Ahluwalia et al., 1984).

6.2.5.2 SOLID TUMOUR MODEL

(a) DETERMINATION OF THE ANTITUMOUR ACTIVITY OF EXTRACT OF P. FLORIDA WHEN ADMINISTERED SIMULTANEOUS WITH TUMOUR CELL INOCULATION

For determining the solid tumour reducing activity of the extract the animals were divided into 4 groups with 6 animals each. Viable DLA cells (1X10^6) in 0.1mlPBS were transplanted subcutaneously into the right hind limb of mice. Methanolic extract of P. florida was administered i.p at doses of 500 and 1000 mg/Kg body weight 24h after tumour inoculation and continued for once in a day for 10 consecutive days (Jones and Janardhanan, 2000). Cisplatin (4mg/Kg, i.p) was used as the standard and the group administered with DLA cells alone as positive control. The development of tumour on animals in each group was measured using vernier calipers twice a week for five weeks and tumour volume was calculated using the formula V = (4/3)πr_1^2 r_2 where r_1 and r_2 are the radii of the tumours. At the end of 5th week animals were sacrificed under diethyl ether anesthesia, tumour extirpated and weighed. The percent inhibition was calculated by the formula (1-B/A) x100 where A is the average tumour weight of the control group and B that of treated group (Chihara et al., 1970).
(b) DETERMINATION OF THE EFFECT OF EXTRACT OF P.SAJOR-CAJU WHEN ADMINISTERED SIMULTANEOUS WITH TUMOUR CELL INOCULATION

To study the effect of methanolic extract of P.sajor-caju against solid tumour in mice were divided into 4 groups of 6 animals each and the experiment was carried out as described earlier using DLA cell lines. The development of tumour on animals of each group was measured using vernier calipers twice a week for five weeks and tumour volume was calculated using the formula $V = \frac{4}{3}\pi r_1^2 r_2$ where $r_1$ and $r_2$ are the radii of the tumours. At the end of 5th week animals were sacrificed under diethyl ether anesthesia, tumour extirpated and weighed. The percent inhibition was calculated by the formula $(1 - B/A) \times 100$ where $A$ is the average tumour weight of the control group and $B$ that of treated group (Chihara et al., 1970).

(c) DETERMINATION OF THE EFFECT OF EXTRACT OF P.FLORIDA WHEN ADMINISTERED AFTER TUMOUR DEVELOPMENT

Antitumour activity of the methanolic extracts of P.florida was tested to find out their effect on developed tumour in mice. Solid tumour was induced as described earlier. After 14 days, animals with tumour volume around $0.40 \pm 0.09$ cm$^3$ were divided into 4 groups of 6 animals in each group. Extract of P.florida (500 and 1000 mg/Kg body weight) was administered intraperitoneally once daily for 10 consecutive days. One group administered with cisplatin (4 mg/Kg body weight, i.p) was taken as standard reference and one group without the administration of drug was kept as positive control. Tumour diameter was
measured using vernier calipers once a week for a period of 3 weeks and volume was calculated. At the end of 5\textsuperscript{th} week, animals were sacrificed, tumour extirpated and weighed. The percent inhibition was calculated as described earlier.

\textit{(d) DETERMINATION OF THE EFFECT OF EXTRACT OF P.SAJOR-CAJU WHEN ADMINISTERED AFTER TUMOUR DEVELOPMENT}

To study the effect of methanolic extract of \textit{P.sajor-caju} against developed tumour the experiment was carried out as described earlier. After 14 days, animals with tumour size around $0.40 \pm 0.09 \text{ cm}^3$ were divided into 4 groups of 6 animals in each group and treatments were done using \textit{P.sajor-caju} extract as described previously. Tumour diameter was measured using vernier calipers once a week for a period of 3 weeks and volume was calculated. At the end of 5\textsuperscript{th} week, animals were sacrificed, tumour extirpated and weighed. The percent inhibition of tumour weight was calculated as described earlier.

6.3 RESULTS

6.3.1 IN VITRO CYTOTOXICITY

The methanolic extracts of \textit{P.florida} and \textit{P.sajor-caju} showed no cytotoxic activity against DLA and EAC cell lines up to a concentration of 1mg/ml when assayed using the trypan blue exclusion method.

6.3.2 ANTITUMOUR ACTIVITY

6.3.2.1 ASCITES TUMOUR MODEL

The methanolic extracts of \textit{P.florida} and \textit{P.sajor-caju} did not show any appreciable antitumour activity against ascites tumour up to a doze of 1000 mg /kg body weight.
6.3.2.2 SOLID TUMOUR MODEL

(i) The methanolic extracts of both the mushrooms possessed significant antitumour activity against DLA induced solid tumour. The extract of *P. florida* significantly reduced the tumour development in a dose dependent manner. The extract at a concentration of 500 mg/Kg prevented the tumour development by 72% with respect to the control and extract at a concentration of 1000 mg/Kg body weight reduced the tumour development by 88%. Tumour reducing effect of the extract at a concentration of 1000mg/Kg body weight was nearly equal to cisplatin at a dose of 4 mg/Kg (Table 6.1).

Similarly methanolic extract of *P.sajor-caju* also significantly reduced the tumour development in a dose dependent manner. The extract at a concentration of 500 mg/mouse prevented the tumour development by 65% with respect to the control. The extract at a concentration of 1000 mg/Kg bodyweight reduced the tumour growth by 82%. Tumour reducing effect of the extract at a concentration of 1000 mg/Kg body weight was nearly equal to cisplatin at a dose of 4 mg/Kg. (Table 6.2)

(ii) The methanolic extract of *P.florida* was also highly effective against developed solid tumour. The extract of *P.florida* at concentrations 500 and 1000mg/Kg body weight when administered for 10 consecutive days after tumour development showed 51.2 and 64% tumour growth regression respectively as compared to control (Table 6.3).

Methanolic extract of *P.sajor-caju* was also highly effective against developed solid tumour. The *P.sajor-caju* at concentrations 500 and 1000
mg/Kg body weight when administered for 10 consecutive days after tumour development showed 50 and 62.5% tumour growth regression respectively as compared to control (Table 6.4). Although methanolic extracts of both *P.florida* and *P.sajor-caju* showed significant tumour reducing activity, *P.florida* extract possessed higher activity than *P.sajor-caju* (Fig 1 and 2).
### Table 6.1: Effect of methanolic extract of *P. florida* on solid tumor

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (mg/Kg)</th>
<th>Tumor Volume (cm³)</th>
<th>Tumor weight (gm)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>---</td>
<td>1.40 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.18 ± 1.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>---</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>4</td>
<td>0.95 ± 0.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.52 ± 0.05&lt;sup&gt;d&lt;/sup&gt;</td>
<td>95.5</td>
</tr>
<tr>
<td><em>P. florida</em></td>
<td>500</td>
<td>0.35 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.19 ± 0.27&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>72.0</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>0.16 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.40 ± 0.15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>88.0</td>
</tr>
</tbody>
</table>

Values are mean ± S.D, n=6 animals
Any two values having a common letter are not significantly different at 5% level, lsd=0.0124 (Tumor volume), lsd=1.098 (Tumor weight)

### Table 6.2: Effect of methanolic extract of *P. sajor-caju* on solid tumor

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (mg/Kg)</th>
<th>Tumor volume (cm³)</th>
<th>Tumor Weight (g)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>---</td>
<td>0.940 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.3 ± 1.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>---</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>4</td>
<td>0.037 ± 0.06&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.58 ± 0.06&lt;sup&gt;d&lt;/sup&gt;</td>
<td>95.1</td>
</tr>
<tr>
<td><em>P. florida</em></td>
<td>500</td>
<td>0.313 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.97 ± 0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>65.0</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>0.370 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.14 ± 0.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>81.9</td>
</tr>
</tbody>
</table>

Values are mean ± S.D, n=6 animals
Any two values having a common letter are not significantly different at 5% level, lsd=0.06596 (Tumor volume), lsd=0.6269 (Tumor weight)
**Table 6.3: Antitumor activity of methanolic extract of *P. florida* on developed tumor**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (mg/Kg)</th>
<th>Volume on 5th week</th>
<th>Weight of Tumor</th>
<th>%Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>---</td>
<td>2.98 ± 0.58a</td>
<td>5.98 ± 0.26a</td>
<td>---</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>4</td>
<td>0.49 ± 0.05c</td>
<td>1.29 ± 0.37d</td>
<td>77.0</td>
</tr>
<tr>
<td><em>P. florida</em></td>
<td>500</td>
<td>1.49 ± 0.29b</td>
<td>2.92 ± 0.12b</td>
<td>51.2</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>0.68 ± 0.15c</td>
<td>2.10 ± 0.20c</td>
<td>64.0</td>
</tr>
</tbody>
</table>

Values are mean ± S.D, n=6 animals. Any two values having a common letter are not significantly different at 5% level, lsd= 0.0896 (Tumor volume), lsd= 0.294 (Tumor weight)

**Table 6.4: Antitumor activity of methanolic extract of *P. sajor-caju* on developed tumor**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (mg/Kg)</th>
<th>Volume on 5th week</th>
<th>Weight of Tumor</th>
<th>%Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>---</td>
<td>3.20 ± 0.60a</td>
<td>6.40 ± 0.36a</td>
<td>---</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>4</td>
<td>0.30 ± 0.08d</td>
<td>1.70 ± 0.29d</td>
<td>76.5</td>
</tr>
<tr>
<td><em>P. sajor-caju</em></td>
<td>500</td>
<td>2.10 ± 0.48b</td>
<td>3.20 ± 0.25b</td>
<td>50.0</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>1.60 ± 0.26c</td>
<td>2.70 ± 0.23c</td>
<td>62.5</td>
</tr>
</tbody>
</table>

Values are mean ± S.D, n=6 animals. Any two values having a common letter are not significantly different at 5% level, lsd= 0.0789 (Tumor volume), lsd= 0.645 (Tumor weight)
Fig 6.1: Comparison of the effect of methanolic extract of P. florida and P. sajor-caju on solid tumour when administered simultaneously
A: 500 mg/Kg body weight, B: 1000 mg/Kg body weight

Fig 6.2: Comparison of the effect of methanolic extract of P. florida and P. sajor-caju on developed tumor
A: 500 mg/Kg body weight, B: 1000 mg/Kg body weight
Fig 6.3: Antitumour activity of methanolic extract of *P. florida* against DLA cell induced solid tumour in mice when treated simultaneously a) Control  b) Methanolic extract (500mg/ Kg body wt)  c) Methanolic extract (1000mg/ Kg body wt)
Fig 6.4: Antitumour activity of methanolic extract of *P.sajor-caju* against DLA cell induced solid tumour in mice when treated simultaneously a) Control  b) Methanolic extract (500mg/Kg body wt) c) Methanolic extract (1000mg/Kg body wt)
6.4 DISCUSSION

The results of the investigations reveal the significant antitumour activity of methanolic extract of *P. florida* and *P. sajor-caju* against solid tumour. However extracts of these mushrooms were not effective against ascites tumour. The antitumour activities of both the mushroom extracts on solid tumour are in a dose dependent manner with no sign of toxicity. The preliminary phytochemical analyses of the methanolic extracts of both the mushroom indicate the presence of protein-bound polysaccharide as the major component of the extract. TLC analysis showed the presence of only traces of flavonoids and terpenes in the extract. The antitumour active fractions in almost all mushrooms were found to be polysaccharides (Gunde-Cimmerman, 1999). In most of the cases, the polysaccharide component belongs to 1-3\(\beta\)D glucans and they have essentially the same structural features. Glucans isolated from the fermentation products of *Pleurotus passeckerianus* were tested in bioassays for their *in vivo* activity against three rodent tumour systems, sarcoma, mammary adenocarcinoma 755, and leukemia L1210 and found to be highly effective against all three models (Jong and Donovick, 1989).

Prevention is one of the rational means of controlling carcinogenesis and nutritional factors can be considered as one of the most significant elements in this endeavour (Doll, 1990, Burstein, 1993). The positive effect of low energy diet specially reduces intake of saturated fats (Willet, 1990). However high fiber intake (Trock et al., 1990) is generally preferred as a preventive measure. From this aspect composition of oyster mushroom with
low energy content, trace amounts of fat, high content of fibrous matter, makes
this fungus an ideal component of dietary prevention of carcinogenesis. The
experimental findings also support this hypothesis. The experimentally proven
antioxidant properties of oyster mushroom also can be considered as an
important evidence to support its antitumour activity (Dreher and Junod, 1996).