CHAPTER 5
ANTICARCINOGENIC ACTIVITY OF THE METHANOLIC EXTRACTS OF PLEUROTUS FLORIDA AND PLEUROTUS SAJOR-CAJU.
5.1 INTRODUCTION

The evolution of an invasive cancer cell from a normal cell, in other words transformation of a normal cell to an invasive cancer cell is called carcinogenesis. Polycyclic aromatic hydrocarbons (PAH), also known as polynuclear aromatic hydrocarbons or polyarenes, constitute a large class of organic compounds. They are formed and released into the environment through natural and man-made sources. The discovery of carcinogenicity of benzo[a]pyrene, dimethyl benz[a]anthracene and other PAH in the mid-1930s was a landmark of the beginning of a new era in biological research to determine the cause and nature of diseases imparted to mammals by this class of compounds. The carcinogenic and mutagenic activities of PAH have been associated with the degree of their nonplanarity (Baum, 1978, DiGiovanni, 1983). Substitution of methyl or other groups into the ring system of PAH can result in molecular distortion from planarity that sometimes translates into more reactivity and carcinogenicity. For example, 7,12- dimethylbenz[a]anthracene is a highly potent carcinogen compared to its parent compound benz[a]anthracene that exhibits much less activity.

Development of cancer is the result of a continuous process that occurs several years during which time damage of numerous regulatory genes eventually may result in premalignant, malignant and then metastatic stages. Cancer is a heterogeneous disease composed of complex genetic changes driving uncontrolled growth and metastatic spread. Reactive oxygen species (ROS) and reactive metabolic intermediates generated from various chemical
carcinogens are known to play an important role in cell damage and in the initiation and progression of carcinogenesis.

During the past 50 years, several major advancements in medicine came from lower organisms such as molds, yeast, and fungi. The first antibiotic was extracted from a fungus. Penicillin, tetracycline and aureomycin, derived from molds, were hailed as wonder drugs for treating infections and communicable diseases. Medicinal mushrooms effective against cancer are known in China, Russia, Japan and Korea as well as USA and Canada (Wasser and Weis, 1999). Our earlier studies aimed to evaluate the antineoplastic activity of this mushroom showed significant antioxidant and anti-inflammatory activities of the methanolic extract of *P. florida* and *P. sajor-caju*. Investigations were carried out to evaluate the anticarcinogenic activities of the methanolic extracts of these mushrooms. The findings are reported in this chapter.

5.2 MATERIALS AND METHODS

5.2.1 ANIMALS

Female Balb/c (25 ± 2g) were used for the study.

5.2.2 PREPARATION OF THE EXTRACT

The extracts were prepared as described in 2.2.1.

5.2.3 DETERMINATION OF THE ANTICARCINOGENIC ACTIVITY OF METHANOLIC EXTRACT OF *P. FLORIDA*

The back of 50 mice was shaved using surgical clippers two days before experiment. Animals with complete hair growth arrest were grouped into 3 groups of 10 animals each and treated as follows. A single dose of 390 nmol
7.12 dimethyl benzanthracene (DMBA) in 0.1 ml acetone was applied for the initiation of tumour (Mimura et al., 1994). After a week 200 µl of 10% croton oil in acetone was applied twice a week for 8 weeks. The methanolic extract of *P. florida* at a concentration of 2 mg and 10 mg in 200 µl acetone/mouse were applied topically 40 minutes before each application of croton oil. Group treated with croton oil alone was served as positive control. Time of tumour induction and number of animals bearing skin papillomas were recorded weekly in each experimental group.

5.2.4 DETERMINATION OF THE ANTICARCINOGENIC ACTIVITY OF *P. SAJOR-CAJU*

To determine the anticarcinogenic activity of *P.sajor-caju*, animals with complete hair growth arrest were grouped into 3 groups of 10 animals each as described above. Time of tumour induction and number of animals bearing skin papillomas were recorded weekly in each experimental group. Methanolic extract of *P. sajor-caju* at a concentration of 2 mg and 10 mg in 200 µl acetone/mouse was used for treatments.

5.3 RESULTS

Topical application of *P.florida* extract on mouse skin after tumor initiation with DMBA resulted in significant protection against skin tumour promotion in a dose dependent manner (Fig 5.1). The induction time was delayed for one week in group of animals treated with the methanolic extract of *P.florida* at a concentration of 2 mg/mouse and two weeks in animals treated with 10mg/mouse (Fig 5.2). The number of tumours in extract treated (10 mg) groups of animals remained constant after 14th and 15th weeks (Fig 5.3).
Topical application of *P. sajor-caju* extract on mouse skin after tumour initiation with DMBA resulted in significant protection against skin tumour promotion in a dose dependent manner (Fig 5.4). The induction time was delayed for one week in the group of animals treated with the methanolic extract of *P. sajor-caju* at a concentration of 2mg/mouse and two weeks in animals treated with 10mg/mouse (Fig 5.5). The number of tumours in extract treated (10mg) groups of animals remained constant after 14th and 15th weeks (Fig 5.6).
Fig 5.1: Effect of methanolic extract of *P. florida* on DMBA induced and croton oil promoted skin papilloma on mice skin a) DMBA + Croton oil b) DMBA + Croton oil + Methanolic extract (2mg) c) DMBA + Croton oil + Methanolic extract (10mg)
Fig 5.2: Effect of methanolic extract of *P. florida* on DMBA induced and croton oil promoted tumor incidence in mouse skin, PF- *P. florida* extract

Fig 5.3: Effect of methanolic extract of *P. florida* on DMBA induced and croton oil promoted tumor incidence in mouse skin, PF- *P. florida* extract
Fig 5.4: Effect of methanolic extract of *P.sajor-caju* on DMBA induced and croton oil promoted skin papilloma on mice skin a) (DMBA + Croton oil b) DMBA + Croton oil + Methanolic extract (2mg) c) DMBA + Croton oil + Methanolic extract (10mg)
Fig 5.5: Effect of methanolic extract of *P.sajor-caju* on DMBA induced and croton oil promoted tumor incidence in mouse skin

PS-*P.sajor-caju* extract

Fig 5.6: Effect of methanolic extract of *P.sajor-caju* on DMBA induced and croton oil promoted tumor incidence in mouse skin. PS-*P.sajor-caju* extract
5.4 DISCUSSION

Significant correlations have been observed between the carcinogenicity of a series of polycyclic aromatic hydrocarbons (PAH) and their covalent binding to mouse epidermal DNA (Brookes and Lawley, 1964, Hoel et al., 1983, Miller, 1978, Pelkonen et al., 1980). Based on extensive evidence accumulated in the last two decades, it is known that PAH must be metabolically activated to electrophilic intermediates, which can bind to DNA and exert their carcinogenic effects (Pelkonen et al., 1980). The metabolic activation of DMBA occurs primarily through the formation of a 3,4 diol 1,2 epoxide (Sims, 1980).

Application of 12-O-tetradecanoylphorbaol-13-acetate (TPA, initiator) stimulates leukocytes and macrophages for the increased consumption of oxygen and in turn generates reactive oxygen species (Athar, 2002). Reactive oxygen species (ROS) derived from these proinflammatory cells are critical component of croton oil, which contains the phorbol ester (TPA) that induces tumour promotion process. Application of TPA also stimulates the infiltration of neutrophils and enhances the myeloperoxidation activity in dermis. All these evidences are suggestive of the fact that reactive oxygen species alter the redox state of cell and may result in the cascade of events related to the progression stage of carcinogenesis.

The carcinogenic activity of PAH is expressed through their biotransformation to reactive intermediates capable of covalently binding DNA.
to induce strand breaks and DNA damage leading to mutation and tumour initiation.

In search of new cancer chemopreventive agents over the past several years, hundreds of natural products have been evaluated. Chemoprevention reduces the risk of carcinogenesis leading to cancer. Recently increased attention has been focused on nonsteroidal anti-inflammatory agents (NSAIDS) which inhibit cyclooxygenase (COX) as potential chemopreventive agents. Many radical scavengers, especially naturally occurring antioxidants have been found to be effective in inhibiting the induction of carcinogenesis by a wide variety of chemical carcinogens (Khajuria et al., 1998).

The experimental results indicates that methanolic extracts of *P. florida* and *P.sajor-caju* impart significant protection against DMBA induced and TPA promoted skin papilloma formation in a doze dependent manner. The findings suggest the profound anticarcinogenic activity of oyster mushroom extract. The significant antioxidant and anti-inflammatory activities of the extracts of these mushrooms might be one of the contributing factors for their anticarcinogenic activity.