SUMMARY AND CONCLUSION
Cancer is the second largest cause of death both in adults and in children. It is as a highly complex, multifactorial disease caused, in part, by endogenous metabolic or other imbalances associated with age or genetic makeup and, in part, by a wide variety of exogenous factors including diet, lifestyle, exposure to ionizing radiation and chemicals of natural or man made origin. The rate of growth of a tumour is a reflection of the aggressiveness of actively dividing cells (the growth fraction), the length of the cell cycle (doubling time), and the rate of cell loss. Variations in these three factors are responsible for the variable rates of tumour growth observed among tumours of differing histologies, as well as among metastatic and primary tumours of the same histology. Major advances in the treatment of cancer have resulted from the recent evolution in medical 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). An ideal drug is one, which selectively effect the cancer cells without being injurious to healthy tissue.

Exposure of DNA to free radicals causes extensive strand breakage and degradation of deoxyribose (Brawn and Fridovich, 1981, Rowley and Halliwell, 1983). The highly reactive hydroxyl radical (OH) can interact with chromatin and result in a wide range of sugar and base-derived products, DNA-protein cross-links and strand breaks. Free radicals are involved in both
the process of aging and the development of cancer (Cross et al., 1987), hence substances which possess antioxidant or free radical scavenging activity have an important role in the prevention of cancer.

Ethyl acetate, methanol and aqueous extracts of *P. florida* and *P. sajor-caju* were evaluated for their antioxidant properties. Methanolic extracts of both the mushrooms showed significant hydroxyl radical scavenging and lipid peroxidation inhibiting activities. It is increasingly realized that majority of diseases/disorders are mainly due to imbalance between pro-oxidant and antioxidant homeostatic phenomenon in the body. A combination of antioxidant molecule is better suited to satisfy diverse biological activity. Therefore antioxidants that impart beneficial effects are presenting a strong basis for the exploitation of their therapeutic potential (Tiwari, 2001). Antioxidants have been demonstrated to play an important role in chemoprevention. The results of the investigations showed that extracts of oyster mushrooms possessed significant antioxidant activity higher than known antioxidant catechin. The findings suggest the therapeutic potentials of *P. florida* and *P. sajor-caju* and possible exploitation of this activity in cancer chemoprevention.

Methanolic extracts of *P. florida* and *P. sajor-caju* were tested against ascites and solid tumour models induced by EAC and DLA cell lines respectively. The extracts significantly reduced solid tumour growth induced by DLA and the effect was in a dose dependent manner. The extracts of *P. florida* and *P. sajor-caju* at a dose of 500 mg/Kg body weight prevented tumour development by 72% and 65% respectively, the reduction was 88% and
82% respectively at a dose of 1000 mg/Kg body weight. These results indicate that the profound antitumour activity of the extracts of these mushrooms. The extracts did not show any appreciable cytotoxic activity. Hence the significant antioxidant activity might be one of the major contributing factors for the antitumour activity of the extracts. Unlike the most commonly used cancer chemotherapeutic agent, the oyster mushroom extract did not show any toxic side effects. This indicated the therapeutic potential of these mushroom extracts. However, both extracts were not active against ascites tumour.

Arthritis and the inflammatory diseases of the large intestine, such as ulcerative colitis and Crohn's disease, are conditions in which oxidative damage has been implicated (Cross et al., 1987). Chronic inflammation is regarded as an essential factor for the progression of neoplastic process. Hence compounds, which are useful in the therapeutic intervention of inflammatory diseases have a major role in delimiting the incidence of human cancers. The methanolic extracts of *P. floridz* and *P. sajor-caju* showed significant protection against acute inflammation induced by carrageenan and chronic inflammation induced by formalin in mouse paw models. Inhibition of inflammation by the extracts at 1000 mg/Kg body weight was over 60% which was markedly higher than the standard reference drug, diclofenac at its therapeutic doze.

Nonsteroidal anti-inflammatory drugs (NSAIDs) have been recently found to have significant use in cancer chemoprevention. NSAIDs have been found to be a potent inhibitor of COX-2. This inhibitory effect is relevant to
cancer chemoprevention because COX catalyses the conversion of arachidonic acid to proinflammatory substances such as prostaglandins, which can stimulate tumour cell growth. Since, extracts of *P. florida* and *P. sajor-caju* possess potent anti-inflammatory activity they might be of significant therapeutic use as cancer chemopreventive agents.

Effect of methanolic extracts of *P. florida* and *P. sajor-caju* against croton oil induced mouse skin edema was evaluated. Active component in croton oil, 12-O-tetradecanoyl-13-phorbol acetate a promoter of mouse skin cancer produces inflammatory cells at the application sites (Athar, 2000). The results showed that the both the mushroom extracts were highly effective against the inflammatory activity of croton oil. The effect was in a dose dependent manner.

Platelets play a crucial part in the blood clotting process by forming a platelet plug. Platelet aggregation has been implicated in the pathophysiology of various clinical disorders. Hence compounds which inhibit platelet aggregation are considered to play an important role in the treatment of several diseases. Methanolic extracts of *P. florida* and *P. sajor-caju* showed significant decrease in platelet aggregation induced by ADP. The metastatic tumour cell lines induce platelet aggregation and subsequent to aggregation, platelet release substances that promote tumour cell growth. Thus inhibition of platelet aggregation has significant importance in antitumour activity. Hence, the platelet aggregation inhibiting activity of the extracts of *P. florida* and *P. sajor-caju* contributes to their antitumour property.
Liver and kidneys are the two important vital organs in animals. Cirrhosis of liver is one of the major health problems in developed countries. Free radicals have been implicated in the pathogenesis of alcohol induced liver injury in humans. CCl$_4$ is metabolically activated to free radicals, when it induces liver injury mainly in perivenular hepatocytes which are rich in drug metabolising microsomal cytochrome P450 system (Sundari et al., 1997). It is well documented that lipid peroxidation plays an important role in CCl$_4$ induced fatty liver and cirrhosis, present studies also support this hypothesis. A significant increase in both the serum and tissue MDA levels was observed in the animals administered with CCl$_4$. The methanolic extracts treatment significantly reduced the increase in MDA levels in a dose dependent manner. The extract also could restore the CCl$_4$ induced decline of status of hepatic antioxidant enzymes. *P. florida* and *P. sajor-caju* extracts also reduced the hepatic marker enzymes such as GOT, GPT and ALP which are elevated after CCl$_4$ administration in a dose dependent manner. The results thus indicate that oyster mushroom extracts possess significant hepatoprotective properties.

Although chemotherapy is a widely accepted method for cancer treatment chemotherapeutic agents show several toxic side effects. Cisplatin is a widely used chemotherapeutic drug for the treatment of various types of cancers. However, nephrotoxicity is one of the dose limiting factors of this drug. Attempts was made to find out the protective effects of methanolic extracts of *P. florida* and *P. sajor-caju* on cisplatin induced nephrotoxicity in mouse model. The extracts of these mushrooms imparted significant protection
against cisplatin induced nephrotoxicity by restoring the decreased antioxidant status, decreased GSH levels and increased lipid peroxidation to almost normal levels in a dose dependent manner. The nephroprotective effect of the extract was also not found to interfere with the antitumour effect of cisplatin. The findings suggest the potential use of the extracts of *P. florida* and *P. sajor-caju* in cancer chemotherapy.

The multi-stage nature of carcinogenesis, originally conceptualized during mouse skin chemical carcinogenesis studies, consisted of the operational concepts of "initiation", "promotion", and "progression" (Trosko and Ruch, 1998). The DMBA induced and croton oil promoted skin papilloma formation is mediated through the generation of free radicals from the inflammatory responses caused by the active component of croton oil, ie. 12-*O*-tetradecanoyl-13-phorbol acetate. Free radicals are involved in the development of cancer (Cross et al., 1987.) They attack many cellular targets including membranes, proteins and nucleic acids, (Cerutti et al., 1994.) and cause structural damage to the cellular DNA. These structural changes manifest as point mutations and chromosomal alterations in cancer-related genes. Hence compounds with the anti-inflammatory and antioxidant activities can effectively inhibit the tumour promotion induced by croton oil. Since the oyster mushroom extracts possessed both antioxidant and anti-inflammatory activities, they were evaluated for the anticarcinogenic activity. Topical application of methanolic *extracts* of *P. florida* and *P. sajor-caju* at a dose of 2 and 10 mg/mouse effectively prevented the croton oil promoted skin papilloma
formation in mouse skin in a dose dependent manner. The maximum effect was at 10 mg concentration. The studies revealed the anticarcinogenic activity of oyster mushroom extracts.

The active compound constituents of mushrooms that show medicinal properties are polysaccharides, lectins, terpenoids, phenol etc (Chang, 1999, Mizuno, 1999). The antitumour components of mushrooms vary in their chemical nature. Later it was found that antitumour active components in almost all mushrooms were polysaccharides. Their derivatives and partially hydrolyzed products were prepared from culture filtrates or by extracting the fruiting bodies (Jong and Donovick, 1989).

Preliminary phytochemical examination of the extracts was carried out. Thin layer chromatography analysis on silica gel G using ethyl acetate: methanol:water (100:16.5:13.5) and chloroform:methanol (95:5) as solvent systems. The chromatograms were examined under UV and also the spots were developed by spray reagents such as 10% antimony trichloride in chloroform and acetic anhydride and H2SO4 (Wagner et al., 1984). Polysaccharide was detected by anthrone reagent (Yemm and Wills, 1954) and phenol sulphuric acid reaction (Dubois et al., 1956). These extracts also responded to anthrone test and phenol sulphuric acid reaction indicating the presence of polysaccharides. These extracts were positive to Lowry’s (1951) test also. TLC analysis showed the presence of only traces of flavonoids in the extract. However, the major constituent of the extract seems to be polysaccharides. Since, extract responded to Lowry’s reagent (Lowry, 1951)
the possibility of the association of protein along with polysaccharide could be ruled out. Hence, the active principle of oyster mushroom extracts might be a protein polysaccharide complex.