African Journal of Biotechnology Vol. 10(37), pp. 7279-7285, 20 July, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.1750
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Anticancer potential evoked by *Pleurotus florida* and *Calocybe indica* using T_{24} urinary bladder cancer cell line

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Accepted 15 April, 2011

Cancer is the second largest non-communicable disease and it has a sizable contribution in the total number of deaths. Cancer of the urinary bladder ranks fourth in men and eighth in women with an alarming increase in cancer patients of this type every year. Tobacco smoking is considered to be one of the causes of bladder cancer and a number of organic chemicals used in the industry are known to be carcinogenic to the bladder. Chemical and dyestuff manufacturers are at particularly high risk of bladder cancer. Edible fungi are used as potential therapeutic agents for the treatment of cancers. This study was employed to find out the therapeutic potential of two medicinal mushroom varieties namely *Pleurotus florida* and *Calocybe indica* against T24 urinary bladder cancer cell lines. The therapeutic potential of the two medicinal mushroom varieties, *P. florida* and *C. indica* against T24 urinary bladder cancer cell lines were determined by the MTT assay and DNA fragmentation assay. The results obtained from the MTT assay and DNA fragmentation assay in this study showed the anti-tumour potential of these two mushroom varieties against T24 bladder cancer cell lines. Thus, the mushroom varieties *P. florida* and *C. indica* can be employed as potential anticancer drugs against bladder carcinoma.

Key words: Bladder cancer, MTT, DNA fragmentation, mushroom, antitumour.

INTRODUCTION

Cancer is a disease that occurs more frequently in later life and the proportion of cancers that occur in the elderly is increasing relative to younger age groups. By 2030, over 70% of all cancers are expected to occur in people aged over 65 years (Edwards et al., 2002). Proposed mechanisms for the increased incidence of cancer in the aging population include an accumulation of genetic and cellular damage, prolonged exposure to carcinogens and fundamental changes in the host environment. Cancer and aging are intimately linked at the most basic level: Convergent mechanisms protect against both aging and cancer (for example, antioxidant defenses), while the pathways regulating cellular proliferation typically exert divergent or opposing effects; specifically, protecting from cancer but promoting aging (Serrano and Blasco, 2007).

It certainly appears that the incidence of bladder cancer is increasing in India. Bladder cancer illustrates well the association between cancer and aging and occurs most commonly in the elderly: The median age at diagnosis is 69 years for men and 71 years for women. Bladder cancer is also the fourth and eighth most common malignancy in men and women, respectively (American Cancer Society, 2009). According to the Delhi cancer registry, in 2003, bladder cancer was the sixth most common cancer, surpassed in frequency only by cancers of the lung, larynx, tongue, prostate and esophagus. Fifteen percent of all tobacco-related cancers are bladder cancers. Curiously, in Northern India, this disease seems to affect younger patients (Rawal, 2008). Bladder cancer,

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arising from the transitional cells of the mucosal urothelium, may present as a noninvasive, papillary tumour protruding from the mucosal surface, or as a solid, nonpapillary tumour that invades the bladder wall and has a high propensity for metastasis. The nonpapillary tumours originate from *in situ* dysplasia.

Bladder cancer's staging, treatment and prognosis depend on how deeply it has invaded urinary bladder (Crawford, 2008). Fortunately, about 80% of patients with non-muscle invasive disease can be successfully treated using the surgery. Historically, two-thirds of patients have tumour recurrence within 5 years. High-grade tumours have a significantly worse prognosis. Both high-grade T1 tumours and carcinoma *in situ* have the potential to progress and even metastasize (Dalbagni, 2007). Patients with invasive bladder cancer require a radical cystectomy. Controversy exists as to whether neoadjuvant or adjuvant chemotherapy improves survival in patients with invasive bladder cancer, despite a number of randomized controlled trials. So far, there are no data to confirm what is the best combination of treatments (neoadjuvant chemotherapy, adjuvant with or without radiotherapy) to treat invasive bladder cancer (Choueiri and Ragavan, 2008). The modest results with current drugs, suggest the urgent need to identify new agents from natural source to treat cancer.

In this regard, edible fungi are frequently used as a therapeutic food having anti-carcinogenic, anti-cholesterolaemic and anti-viral properties and also prophylactic properties with regard to coronary heart disease and hypertension (Bobek et al., 1995a, b; Mattila et al., 2000). Mushrooms also display certain medicinal properties and have a good biological efficiency (60 to 70%) under optimum conditions. The moisture content of fresh mushrooms varies from 86 to 93% depending upon their environmental conditions and protein content varies from 2 to 3%. Due to its higher moisture content, they are highly perishable in nature and self life varies from 1 to 2 days. Actually, world trade in mushrooms is as big as the trade in coffee. Mushroom polysaccharides support some or all of the major systems of the body, including nervous, hormonal and immune systems as well as regulatory functions. The polysaccharides from mushrooms do not attack cancer cells directly, but produce their antitumour effects by activating different immune response in the host (Wasser, 2002).

The scientific community, in searching for new therapeutic alternatives, has studied many kinds of mushrooms and found variable therapeutic activites such as anticarcinogenic, anti-inflammatory, immune-suppressor and antibiotic activity (Turkoglu et al., 2006). Although, the isolation process, structural characterization and antitumour activity of mushroom polysaccharides have been extensively investigated in the past three decades, the relationship between the antitumour activity and the chemical composition as well as the high order structure of their active components is still not well established. Recent literature showed that, medicinal mushrooms occurring in south India namely *Pleurotus florida* and *Calocybe indica* possessed profound antioxidant, antibacterial and antitumour activities. In this regard, in 2007, Ajith and Janardhan investigated the anticancer properties of some Indian Mushrooms (Ajith and Janardhan, 2007). These studies are still in progress in many laboratories and the role of polysaccharides as antitumour agent is especially under intense debate (Zhang et al., 2006). The T24 cell line has been established from a highly malignant grade III human urinary bladder carcinoma (Bubenak et al., 1973). This cell line can be easily grown *in vitro* and has been extensively used to evaluate the therapeutic effects of several anticancer drugs.

So, the current investigations will assist the confirmation of the utility of the bioactive compounds from the aforementioned said mushrooms namely *P. florida* and *C. indica*.

MATERIALS AND METHODS

Collection and preparation of sample

Mushroom samples were collected from Blue hill mushroom producers and the sample was preserved in the Department of Biochemistry, by Dr N. G. P. Arts and Science College, Coimbatore. The mushrooms were shade dried and made into a coarse powder. The coarse powder was extracted using ethanol for 72 h in a Soxhlet's apparatus. The ethanol was evaporated and the extract was concentrated and was used for the assay.

Collection of cell T24 bladder transitional cell carcinoma cell lines

Cell line T24 was provided by National Centre for Cell Sciences, Pune. Cells were grown as a monolayer in complete RPMI (RPMI-1640 medium supplemented with 10% fetal calf serum, 100 U/ml penicillin and l00 U/ml streptomycin), in a humidified atmosphere with 7% CO_2 93% air at 37°C. T24 cell lines were used as a bladder tumor model to study the anticancer effect of the mushroom extracts.

MTT assay

The antitumor potential of the mushroom extracts were analysed by MTT assay (Mossmann, 1983), measurement of cell viability and proliferation forms the basis for numerous *in vitro* assays of a cell population's response to external factors. The reduction of tetrazolium salts is now widely accepted as a reliable way to examine cell proliferation. The yellow tetrazolium MTT (3- (4, 5-dimethylthiazolyl-2)-2,5- diphenyltetrazolium bromide) is reduced by metabolically active cells, in part by the action of dehydrogenase enzymes, to generate reducing equivalents such as NADH and NADPH. The resulting intracellular purple formazon can be solubilized and quantified by spectrophotometric means.

DNA fragmentation assay

The antitumor potential of the mushroom extracts were analysed by DNA fragmentation assay (Gong et al., 1994). DNA fragmentation assay provides a method for DNA separation of fragmented and

Table 1. MTT Cytotoxicity assay of *P. florida* AND *C. indica.*

S/N	Concentration of extract (µg/ml)	Percentage inhibition	
		Pleurotus florida	***Calocybe indica***
1	10	39 ±2.36	34±1.46
2	25	46±1.53	44±3.15
3	50	60±1.82	57±2.63
4	100	71±3.91	68±2.80
LC 50 value		35.67	36.13

Values represented as Mean ± SD. LC 50 to 50% lethal concentration.

intact DNA fractions and for their analysis by agarose gel electrophoresis. In apoptotic cells, specific DNA cleavage becomes evident in electrophoresis analysis as a typical ladder pattern due to multiple DNA fragments. This protocol is simple and generally able to provide good results and it is only a qualitative method of DNA analysis.

Statistical analysis

The study was carried out in triplicates. The statistics used was standard deviation for testing the consistency of the results obtained.

RESULTS

In this study, various concentrations of the ethanolic extract of *P. florida* and *C. indica* (10, 25, 50 and 100 µg/ml) inhibited the growth of the urinary bladder carcinoma. Cell line T24 cell to a good extent and the percentage of inhibition brought about by the ethanolic extract of *P. florida* (39±2.36, 46±1.53, 60±1.82, 71±3.91) and *C. indica* (34±1.46, 44±3.15, 57±2.63, 68±2.80) are shown in Table 1; Figures 1 and 2. The percentage of inhibition increased in a dose dependent manner in both the extracts.

DNA fragmentation assay

The concentration of the ethanolic extract of *P. florida (*35 µg/ml) and *C. indica (*36 µg/ml*)* which showed 50% inhibition of tumour cell line growth as obtained from the MTT assay is used for the DNA fragmentation assay. Fragmentation of genomic DNA to high molecular weight (180 to 200 kb) fragments is a characteristic of the early event in apoptosis and may represent the committed step of the process. Apoptosis or programmed cell death represents a physiological form of cell death that occurs during development and in the mature animal.

DISCUSSION

Peng et al. (2005) reported that the T24 cells were found very susceptible to *Antrodia camphorata* extract which arrested the T24 cell growth at a concentration of 50 µg/ml, assayed through MTT assay. The MTT assay is a rapid and sensitive spectrophotometric assay for determining viability in monolayer culture cell lines. The assay involves the conversion of the tetrazolium salt MTT by viable proliferating cells to an insoluble product, purple formazon. This study supported the present work. The result from this study revealed that the ethanolic extracts of *P. florida* and *C. indica* could arrest the growth of the cancer cell line T24.

Cancer has a scourge on the human population for many years. Although, numerous advances have been made in prevention, diagnosis and treatment of the disease, it still continues to torment mankind (Hanahan and Weinberg, 2000).

Chemoprevention (ingestion of chemical agents that reduce the risk of carcinogenesis) is one direct way to reduce this toll. Mushrooms that are useful against cancers of the stomach; esophagus, lungs, etc. are known in China, Russia, Japan and Korea, as well as in the United States and Canada. Approximately, 200 species of mushrooms have been found to markedly inhibit tumour growth; although, most mushroom-origin antitumour substances have not been clearly identified. Polysaccharides from mushrooms do not attack cancer cells directly, but activate different immune responses in the host. Most of the active polysaccharides isolated from mushrooms can be classified as dietary fiber. β-Glucans and chitinous substances with carcinostatic activity, contained primarily in the dietary fiber of mushrooms, absorb hazardous materials.

They prevent absorption and hastening excretion and may help prevent cancer of the colon and rectum. Lectins have been isolated and purified from several higher basidomycetes. These materials can be used for affinity chromatography, for diagnosis of cancer cells or as specific binding moieties for targeted cancer therapy (Wasser and Weis, 1999). With this background knowledge as supportive evidence, this study revealed the *in vitro* antitumour activity of the ethanolic extracts of *P. florida* and *C. indica.*

Thus, from the MTT analysis carried out in the urinary bladder, tumour cell line T24 revealed the ethanolic extract of *P. florida* and *C. indica* could effectively reduce

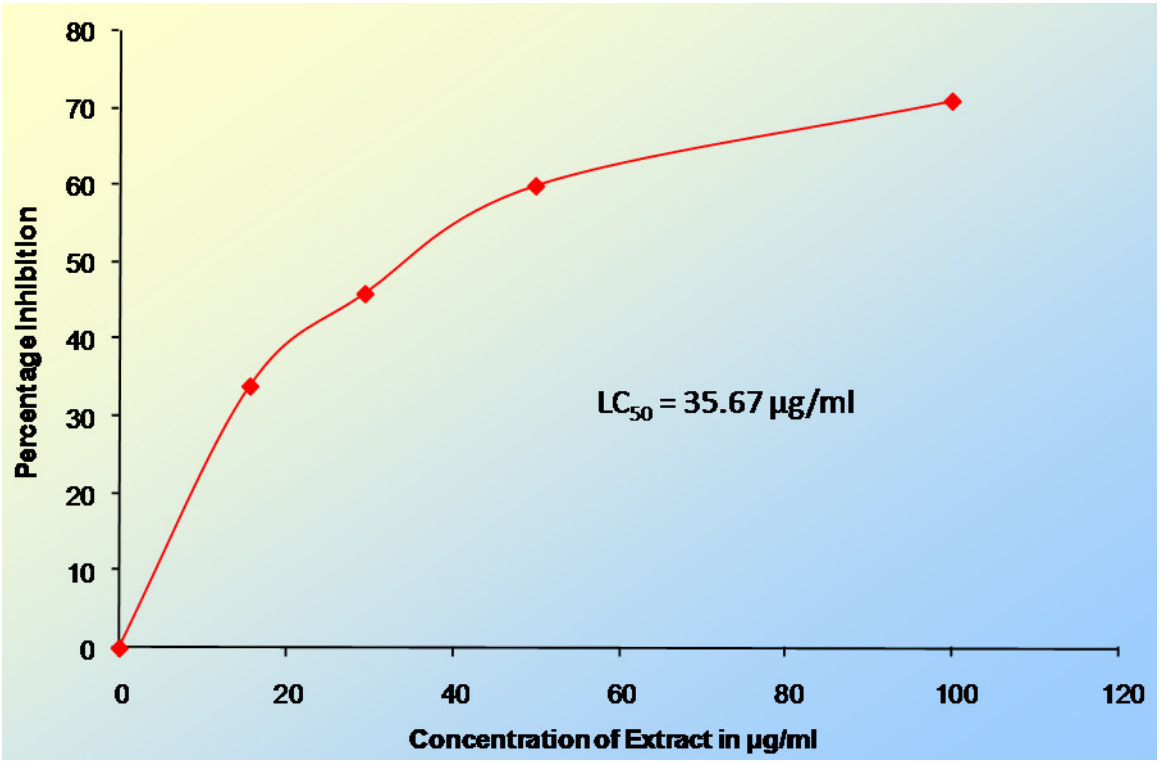

Figure 1. MTT cytotoxicity assay of *P. florida.*

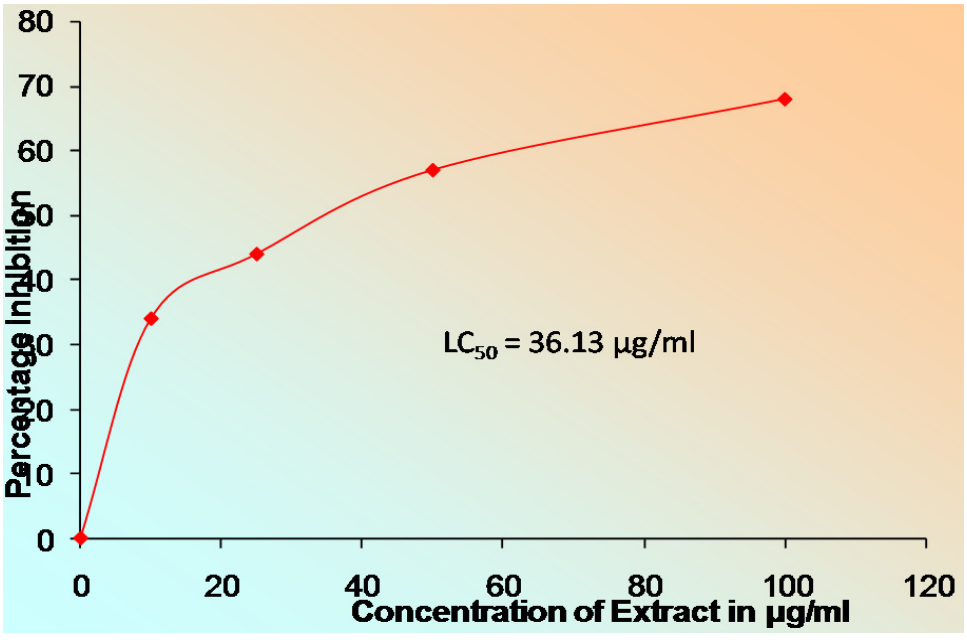

Figure 2. MTT cytotoxicity assay of *C. indica.*

the growth and multiplication of the tumour cell and ultimately suppress the growth of the tumour cells. The anticarcinogenic effect of the ethanolic extract of *P. florida* and *C. indica* may be due to the rich antioxidant content and the presence of secondary metabolites like flavonoids and phenols in them. Foods rich in antioxidants

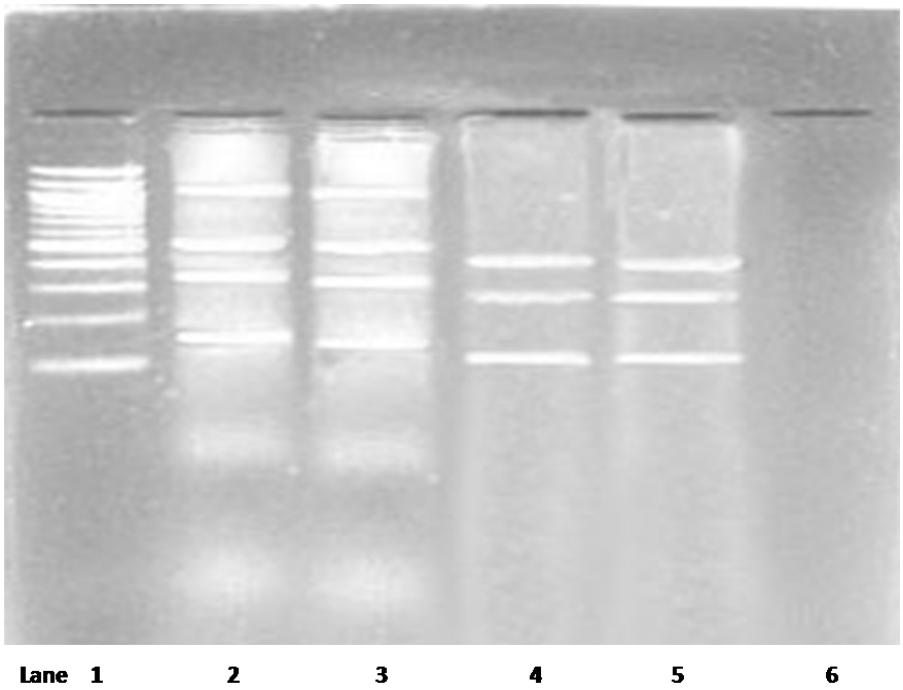

Figure 3. DNA fragmentation induced by the ethanolic extract of *P. florida* and *C. indica.* Lane 1, Marker DNA; lane 2 to 3, T24 cells + ethanolic extract of *P. florida* (35 µg/ml); lane 4 to 5, T24 cells + ethanolic extract of *C. indica* (36 µg/ml); lane 6, untreated T24 cells.

have been shown to play an essential role in the prevention of cardiovascular diseases, cancer, neurodegerative diseases, inflammation and problems caused by cell and cutaneous aging (Pratt, 1992). Most people if they think about mushrooms at all consider them a food with no particular value beyond taste. As a significant source of protein, fiber, several minerals, B vitamins and ascorbic acid, mushrooms are actually a healthy addition to the diet. They also have uses beyond nutrition, having numerous medicinal quantities (Meletis, 2005).

Agarose gel electrophoresis was used to evaluate whether high molecular DNA fragmentation was a feature of the cellular response of the tumour cell to the ethanolic extract of *P. florida* and *C. indica.* The internucleasomal DNA was detected on agarose gel as a ladder of DNA fragment (Figure 3) and this confirmed that the cytotoxic effect of the mushroom extracts is mediated via apoptosis. It is emphasized that, there was a close relationship between apoptosis and proliferation in malignant neoplasm cells. A characteristic DNA laddering was noticed only in tumor cell line treated with the ethanolic extract of *P. florida* and *C. indica.* This pattern was not observed in the control cells and the cells grown in ethanolic extract of *P. florida* and *C. indica* free medium. The data reported here demonstrated the lethal injury to carcinoma cells via activation of the cell death by both the ethanolic extract of *P. florida* and *C. indica.*

Emerging research suggests that, mushrooms and mushroom extracts may have potent anticancer activity, for both breast and prostate cancer. Research shows that, 30 to 35% of all cancers can be prevented by eating well, being active and maintaining a healthy body weight (Canadian Cancer Society). As fresh mushrooms are low in calories and fat, as well as being versatile and great-tasting, they are a good addition to a healthy eating pattern. They are satisfying as well, which may help keep the calories in check and weight at a healthy level. The Canadian cancer society recommends choosing 5 to10 servings of vegetables and fruit every day to reap the benefits of their disease-fighting antioxidants and phytochemicals.

Mushrooms offer nutrients such as β-glucans and conjugated linoleic acid, compounds that are currently being studied for their chemopreventive potential. Mushrooms are a valuable source of fiber, both insoluble (cellulose, lignin, chitin) and soluble the main constituents of which are β-glucans and chitosans (Sadler, 2003). Most clinical evidence for antitumour activities comes from the commercial polysaccharides isolated from shitake (*Lentinus edodes*) and maitake (*Grifola frondosa*) mushroom. The authors suggest that consuming 3.5 ounces (100 g) of mushrooms per day would help suppress breast tumour growth in women. However,

much more research, including human studies, needs to be done before any specific recommendations can be made. Numerous bioactive polysaccharides or polysaccharide-protein complexes from medicinal mushrooms are described that appear to enhance innate and cell-mediated immune responses and exhibit antitumour activities in animals and humans. Stimulation of the host immune defense systems by bioactive polymers from medicinal mushrooms has significant effects on the maturation, differentiation and proliferation of many kinds of immune cells in the host. Numerous reports have documented the ability of PSK and PSP to activate cellular and humoral components of the host immune system. In addition, these polysaccharides have been shown to inhibit the growth of tumour cell lines and to have *in vivo* anti-tumour activity (Tzianabos, 2000).

The specificity of an anticancer compound against cancer cell depends on the proliferation rates of the treated cells. Cancer cells proliferate more rapidly than normal cells, which make them more sensitive to drug treatment. Plant-derived anticancer agents act by entering with cancer cell proliferation or by the induction of apoptosis in tumour cells (Ferreira et al., 2002). The advantage of apoptosis inducing anticancer drugs is that the apoptotic bodies formed can be scavenged by the human body's immune system without inducing an inflammatory response (Frink and Cookson, 2005). In addition to apoptosis induction, another common effect shared by many anticancer drugs is the introduction of cell cycle arrest. Cell cycle arrest has been found in cells exposed to a variety of stimuli, including irradiation (Holgersson et al., 2005), microtubule stabilizing agents (Bhalla, 2003) and topoisomerase inhibitors (Tolis et al., 1999; Potter and Rabinovitch, 2005).

The anti-tumour activity of medicinal mushrooms has been evaluated in Japan for prevention of esophageal, gastric and lung cancers with promising results (Ng, 1998). In phase II and phase III trials in China, PSP significantly enhanced immune status in 70 to 97% of patients with cancer of the stomach, esophagus, lung ovary and cervix. In these studies, PSK and PSP increased the number of immune cells and facilitated dendritic and cytotoxic T-cell infiltration of tumours. The polysaccharides were well-tolerated and compatible with chemotherapy and radiation treatment. Based on the study, the ethanolic extract of *P. florida* and *C. indica* revealed the potent antitumour effect.

Conclusion

The result from the MTT assay showed that the ethanolic extracts of *P. florida* and C*. indica* at a concentration of 35 and 36 µg/ml, respectively, could prevent the proliferation of the tumour cells. The DNA fragmentation assay also clearly indicates that the mushroom extracts could arrest the growth of tumour cell and could induce apoptosis in the tumour cells without the involvement of the inflammatory reactions.

REFERENCES

Ajith TA, Janardhanan KK (2007). Indian Medicinal Mushrooms as a Source of Antioxidant and Antitumor Agents. J. Clin. Biochem. Nutr. 40: 157-162.

American Cancer Society [online] (2008); Cancer Facts & Figures 2008 [http://www.cancer.org/downloads/STT/2008CAFFfinalsecured.pdf] (accessed 21 January 2009)

Balmain A, Gray J, Ponder B (2003). The genetics and genomics of cancer. *Nature Genet* . 33: 238.

Bhalla KN (2003). Microtubule – targeted anticancer agents and apoptosis. *Oncogene.* 22(56): 9075-9086.

Bobek P, Galbavy S (1999). Hypocholesterolemic and antiatherogenic effect of oystermushoom in rabbit. *Nahrung,* 43(5):339-342.

Bobek P, Ozdyn L, Kuniak L (1995). The effect of Oyster (*Pleurotus ostreatus)* its ethanolic extract and extraction residues on cholesterol levels in serum lipo proteins and liver of rat. *Nahrung,* 39 (4):98-99.

Bubenak J, Baresova¡ M, Viklicka V, Jakoubkova J, Sainerova H, Donner J(1973). Established cell line of urinary bladder carcinoma (T24) containing tumour-specific antigen. Int. J. Cancer. (11):765-773.

Choueiri T, Raghavan D (2008). Chemotherapy for muscle-invasive bladder cancer treated with definitive radiotherapy persisting uncertainties. Nat Clin Pract Oncol . 5:444-454.

Crawford J (2008). The origins of bladder cancer. *Lab Inves.* 88: 686-693. doi: 10.1038/labinvest.2008.48.

Dalbagni G (2007). The Management of Superficial Bladder Cancer. Nat. Clin. Pract. Urol. 4: 254-260.

Edwards BK, Howe HL, Ries LA, Thun MJ, Rosenberg HM, Yancik R, Wingo PA, Jemal A, Feigal EG (2002). Annual report to the nation on the status of cancer, 1973-1999, featuring implications of age and aging on U.S. cancer burden. Cancer. 94(10): 2766-2792.

Ferreira CG, Epping M, Kruty FA, Giaccone G (2002). Apoptosis: target of cancer therapy. Clinical Cancer Research, 8(7): 2024-2034

Frink SL, Cookson BT (2005). Apptosis, pyroptosis and necrosis, mechanistic description of dead and dying eukaryotic cells. Infection and Immunity, 73(4): 1907-1916.

Ghobrial IM, Witzig TE, Adjei AA (2005). Targeting apoptosis pathways in cancer therapy. CA Cancer J. Clin. 55(3): 178-194

Gong J, Traganos F, Darzynkiewicz Z (1994). A Selective Procedure for DNA Extraction from Apoptotic Cells Applicable for Gel Electrophoresis and Flow Cytometry. Analyt Biochem. 218: 314-319.

Hanahan D, Weinberg RA (2000). The hallmarks of cancer. *Cell* ; 100: 57-70.

Holgersson A, Heiden T, Castro J, Edgren MR, Lewensohn R, Meijer AE (2005). Different G2/M accumiation in M05aJ and M05qk cells after exposure to DNA double – strand break – inducing agents. Int. J. Radiation Oncol. Bio. Phy. 61(3): 915-921.

Lynch CF, Cohen MB (1995). Urinary system. Cancer. 75(1): 316-329.

Mattila P, Suonpaa K, Pirron V (2000). Functional properties of edible mushrooms. Nutr., 16: 694-696.

Meletis CD, Barker JE (2005). Medicinal Mushrooms. A selective overview. Alternative and complementary Therapies. 11(3): 141-145.

Mossmann, T (1983). Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferiation and Cytotoxicity Assays. J. Immunol. Meth. 65:55-63.

Ng TB, (1998). A review of research of on the protein bound polysaccharide (polysaccharopeptide, PSP) from the mushroom *Coriolus versicolor* (Basdiomycetes). Polypraceae Gen. Pharmacol. 30: 1-4.

Peng CC, Chen KC, Peng RY, Su CH, Li HMH (2005). Human urinary bladder cancer T24 cells are susceptible to the *Antrodia camphorate* extracts. Cancer letters, 243 (1): 109-119.

Potter AJ, Rabinovitch PS (2005). The cell cycle phases of DNA damage and repair initiated by topoisomerase II-targeting chemotherapeutic drugs. Mutation Research; 572(1-2):27-44.

Pratt DE (1992). Natural Antioxidants from plant material in phenolic

compounds in foods and their effects on health II: Antioxidants and caner prevention I. In: Hang M, Ho C, Lee C, eds. *ACS symposium series 507.* Washington DC: American Chemical Society, 54.
Rawal S (2008). Bladder cancer: A difficult problem?. Indian J. Urol. 24:60.
Sadler M (2003). Nutritional properties of edible fungi. British Nut. Foundation Nutr. Bulletin, 28: 305-308.
Serrano M, Blasco MA (2007). Cancer and ageing: convergent and divergent mechanisms. *Nat. Rev. Mol Cell Biol* . 8: 715–722
Tolis C, Peters GJ, Ferreira CG, Pinedo HM, Giaccone G (1999). Cell cycle disturbances and apoptosis induced by topotecan and gemeitabine on human lung cancer cell lines. Euro. J. Cancer. 35(5): 796-807.
Turkoglu A, Kivrak I, Mercan N, Dure Me, Gezer K, Turkoglu H (2006). Antioxidant and antimicrobial activities of Morchella conica pers. Afr. J. Biotechnol . 5(11):1146-1150.
Tzianabos A (2000). Polysaccharide immunomodulators as therapeutic agents: structural aspects and biologic function. Clinical Microbiol. Rev. 13: 523-533.
Wasser SP, Weis AL (1999). Medicinal properties of substances of occurring in Higher Basidiomycetes Mushrooms; Current Perspectives (Review). Int. J. Medicinal Mushrooms, 1: 31-62.
Wasser SP (2002). Medicinal mushroom as a source of antitumour and immunomodulating polysaccharides. Appl. Micorbiol. Biotechnol. 60(3): 258-274.
Zhang M, Cui SW, Cheung PCK, Wang Q (2006). Antitumour polysaccharides from mushrooms; a review on their isolation process, structural characteristics and antitumour activity. Trends in Food Science and Technology, 18:4-19.