CHAPTER – I

1.0 INTRODUCTION

Over the past two decades, cancer incidence rates continue to increase. Among women, under the age of 40, approximately 1 in 47 will develop cancer. Cancer is one of the leading causes of death across the globe. It is estimated that about 13 million people were diagnosed with cancer and about eight million men and women died of cancer in the year 2010. A total of 1,596,670 new cancer causes and 5,71,950 deaths are projected to occur in United States in 2011 (Siegel et al, 2012).

Cancer, a major killer disease is a complex sequence starting from diagnosis till therapy. Cancer can arise from any type of living cells of the body. The onset and development of cancer is a multistep process that is controlled by many genes that regulate the biochemical reactions undergoing at the molecular level inside the body tissues (Rajshri M Navalakhe and Tarala D Nandedkar, 2007).

The body is made up of hundreds of millions of living cells. Normal body cells grow, divide and die in an orderly fashion. During the early years of a person’s life, normal cells divide faster to allow the person to grow. After the person becomes an adult, most cells divide only to replace worn out or dying cells or to repair injuries. Cancer begins when cells in a part of the body start to
grow out of control. Cancer cell growth is different from normal cell growth. Instead of dying, cancer cells continue to grow and form new, abnormal cells. Cancer cells can also invade to other tissues, something that normal cells cannot do (American Cancer Society, 2012).

There have been significant improvements in diagnosis and treatment of several cancers, particularly an increased survival rate for cancer patients who are diagnosed at early stages. Regardless, most cancers were diagnostic, surgical and therapeutic procedures have not yet evolved, cancer elimination and prevention are still a major challenge. For many decades, cancer drug development strategies led to several promising drugs, some of which have proven to be successful in cancer prevention and treatment. Despite the advances in the drug development, clinical intervention options are still limited for many types of human cancers (Morgan TR, 2011).

Bladder cancer is the 10th most common cancer worldwide, with the highest rates reported in Europe, North America and Australia and accounting for an estimated 2,61,000 new cases diagnosed and 1,15,000 deaths each year by comparison, relatively low rates are found in the Far Eastern countries. In Europe, bladder cancer is the 5th most commonly diagnosed cancer type and the 9th leading cause of cancer mortality. It affects men more frequently than women. (Larsson et al, 2008).
Typical of solid tumors, bladder cancer incidence increases with age. Tumors of the bladder rarely occur before the age of 40 – 50, arising most commonly in the seventh decade of life. The median ages at diagnosis are 69 years for men and 71 for women. Histologically, most cases of bladder cancer are transitional cell carcinomas (90 %), 70 % of these are superficial and papillary subtypes. The less common types are squamous cell carcinoma (3 – 5 %), adenocarcinoma (0.5 to 2 %), small cell carcinoma (less than 0.5 %) and sarcoma, carcinosarcoma/sarcomatoid tumours, paraganglioma, melanoma and lymphoma (less than 0.1 %). Haematuria, i.e., frequent urination and pain during urination, are the most common symptoms of bladder cancer (Shariat et al 2009).

Urinary bladder cancer occurs in all countries around the world and it is the fifth most common cancer in the United States. Bladder cancer is the second most common malignancy of the genitourinary tract worldwide after prostate cancer. Globally, approximately 3,36,000 new cases of bladder cancer occurred in 2000, two-thirds of which were in developed countries. Bladder cancer is the fourth most common cancer in males and the ninth most common cancer in females (Yavari et al., 2009).

Bladder cancer is almost three to four times more common in men than in women in most populations. This reflects the more frequent exposure of men to tobacco smoke and occupations that imply contact with certain chemicals,
such as aromatic amines, which are the two major recognized risk factors for bladder cancer (Negri and Vecchia, 2001). About 65% of bladder cancer in men and 30% of female cases in some developed countries are exposed to smoking. Cigarette smoking is the most risk factor for inducing bladder cancer (Scélo and Brennan, 2007). Several studies showed that smokers were susceptible to a 209-fold to 308-fold increase in the risk for bladder cancer (Zeegers et al., 2004).

Other risk factors mainly include workers exposed to some toxic agents, less fluid intake and consumption of fruits and vegetables, urinary tract disease and elderly persons. About 1,32,000 people each year die from bladder cancer worldwide and the mortality rate is 10 per 100,000 for males and 2 per 100,000 for females. These rates nearly double for developed countries. Hematuria is the most common presentation of patients with bladder cancer. Other symptoms such as frequency, urgency and dysuria may be the initial presentation of bladder cancer (Anderson and Naish, 2008).

Transitional cell carcinoma of the bladder is a significant health problem worldwide. Many transitional cell carcinoma (TCC) cases are superficial and may be treated with endoscopic resection. However, the recurrence rate is high for tumors treated with resection alone, which has led to the use of adjuvant therapy with intravesical agents (Holmang, S, 2000).
The study indicate that directing against Phosphatidylinositol dependent kinase 1 (PDK1) through DTCM-glutarimide (3-[(dodecylthiocarbonyl) methyl]glutarimide) treatment could be useful to prevent cancer progression and abnormal tissue dissemination of advanced bladder carcinoma. (Maria et al., 2012).

Joao et al., (2012), demonstrates that application of COS3 has a preventive effect on bladder cancer appearance, as well as it can be successfully used as a curative beneficial ingredient, dependent on the concentration. Tian et al., (2008), findings showed the anticancer efficacy of curcumin against human bladder cancer cells invitro and invivo.

Rosario Pinto et al. (2009) reported that Sirolimus has an anti-proliferation effect on the T24 bladder carcinoma cell line. The information from our results is useful for a better understanding sirolimus’s anti-proliferative activity in the T24 bladder cancer cell line.

Polymers are macromolecules composed of repeating structural units of monomers connected by covalent chemical bonds and this process is known as polymerization. There are many types of polymers including natural and synthetic moiety. Natural polymers such as proteins (collagen, silk and keratin), carbohydrates (starch, glycogen) are widely used materials for conventional and novel dosage forms. These materials are chemically inert, nontoxic, less expensive, biodegradable, eco-friendly and widely available (Malviya et al., 2010).
Chitosan receives a lot of attention because of its numerous desirable qualities and that it is produced from chitin, which is the second most abundant biopolymer in the world. The growing interest in chitosan is due to its biocompatibility, biodegradability, antibacterial properties, affinity for many proteins, and anti-oxidative properties (Liu et al., 2012). It can be used as a flocculent, clarifier, chromatography column matrix, gas-selective membrane, plant disease resistant promoter, anti-cancer agent, wound healing promoting agent and antimicrobial agent (Ocloo et al., 2011)

The development of new applications for chitosan and its derivative is mainly due to the fact that these are renewable source of natural biodegradable polymers and also due to chitin and its derivative are the most abundant natural polymers. The main factors which stimulated the interest in chitosan utilization in various fields from fertilizers to pharmaceuticals are its versatility, economical and easily availability. Chitosan is no longer just a waste by-product from the seafood processing industry. This material is now being utilized by industry to solve problems and to improve existing products, as well as create new ones. Chitosan is modified natural, biodegradable, biocompatible, nontoxic, as well as linear nitrogenous polysaccharides, a basic polysaccharide homo-polymer (Malviya, et al, 2010).

Chitosan have attracted considerable interest due to their biological activities, namely, antimicrobial (Zhao and Xia, 2006), hypocholesterolemic (Liao

An antitumor activity of chitosan has been claimed by inhibition of the growth of tumor cells mainly due to an immune stimulation effect. Studies carried out using mice that had ingested low-molecular weight chitosan revealed significant antimetastatic effects of chitosan against Lewis lung carcinoma. Partially deacetylated chitin as well as chitin with a carboxymethyl group have also been effective to demote tumor progression (Kim and Rajapakse, 2005).

Chitosan has shown a significant scavenging capacity against different radical species, the results being comparable to those obtained with commercial antioxidants. Samples prepared from crab shell chitin with degree of deacetylation of 90, 75 and 50% where evaluated on the basis of their abilities to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, hydroxyl radical, superoxide radical and alkyl radical. The results revealed that chitosan with higher degree of deacetylation exhibited the highest scavenging activity (Park P.J, 2004).

The antimicrobial activity of chitin, chitosan, and their derivatives against different groups of microorganisms, such as bacteria, yeast, and fungi, has received considerable attention in recent years. Two main mechanisms have been suggested as the cause of the inhibition of microbial cells by chitosan. The interaction with anionic groups on the cell surface due to its polycationic nature
causes the formation of an impermeable layer around the cell which prevents the transport of essential solutes. It has been demonstrated by electron microscopy that the site of action is the outer membrane of gram negative bacteria. The permeabilizing effect has been observed at slightly acidic conditions in which chitosan is protonated but this permeabilizing effect of chitosan is reversible (Helander et al, 2001).


Qingsong Xu et al (2008), demonstrated the chitosan induce human hepatocellular carcinoma cells (SMMC 7721 cells), regulate the Bax expression, trigger the cells a start up of the apoptosis program. Theresa et al (2006), studied that chitosan treatment decreases SK-OV- 3 cell proliferation and causes morphological changes in this cell line. Zakir Hossain and Koretaro Takahashi (2008), demonstrated that modified chitosan significantly inhibited cell proliferation, induced apoptosis in HT-29 cell line.
Jiangli Dou et al (2011), conclude that both the extrinsic and intrinsic pathways participate in the apoptosis of HL-60 induced by oligochitosan. Furthermore, oligochitosan arrest cell cycle at G0/G1 phase through upregulating the expression of p21. Galectin-9 is involved in the apoptosis and plays a synergistic effect with oligochitosan.

Although, the antitumor effect of chitosan has been already demonstrated in the above mentioned cell lines, the antioxidant and antitumor effect of chitosan on T24 Urinary bladder cancer cell line has not yet investigated so far. The antimicrobial activity of chitosan oligomers have been demonstrated by Roller and Covil, 1999; Lie et al., 2001). Further, The antimicrobial effect of chitosan cream base pharmaceutical preparation has not been studied.

Therefore, the present study was undertaken to screen the antioxidant, antimicrobial and antiproliferative efficacy of chitosan and its pharmaceutical preparations on T24 urinary bladder cancer cell line. The study was also carried out in benzidine induced urinary bladder cancer in swiss albino mice to evaluate the antioxidant effect of chitosan invivo. The present study was carried out in the following phases.
PHASE – I – Screening of invitro antimicrobial and antioxidant efficacy of chitosan and its pharmaceutical preparations.

1. To evaluate the film forming properties of chitosan in Pharmaceutical preparations.
2. To demonstrate the antimicrobial activity of chitosan and its semi solid preparations.
3. To manifest the wound healing activity of chitosan in semi solid preparations.
4. To evaluate the invitro antioxidant efficacy of the chitosan.

PHASE II – Screening of invitro antiproliferative efficacy of chitosan in T 24 Urinary bladder cancer.

1. Invitro cytotoxicity assay (MTT Assay) of chitosan in T24 Urinary bladder cancer cell line
2. Apoptosis by DNA fragmentation study of chitosan in T24 Urinary bladder cancer cell line
3. Cell cycle distribution analysis of chitosan in T24 Urinary bladder cancer cell line

PHASE III – Screening of invitro antitumor efficacy of chitosan in benzidine induced bladder cancer in swiss albino mice.

1. To screen the antitumor potential of chitosan against benzidine induced Urinary Bladder Cancer in Swiss albino mice and histopathology study.