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Research Article

Comparative Study of Cytotoxic Efficacy of Chitosan and Cyclophosphamide against T24 Human Urinary Bladder Cancer Cell Line

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ABSTRACT

A comparative study of cytotoxic efficacy of chitosan and cyclophosphamide by evaluating cell viability in T24 human urinary bladder cancer cell line.Treatment of T24 cells with increasing concentration of Chitosan and cyclophosphamide led to a concentration dependent decrease in cell immigration. The present study proves that Chitosan has cytotoxic effects on T24 human bladder cancer cell line, which was compared with cyclophosphamide drug.

Keywords: Chitosan, T24 cell line, MTT assay, Cyclophosphamide.

INTRODUCTION

Cancer is one of the leading causes of death across the globe. It estimated that about 13 million people were diagnosed with cancer and about eight million men and women died of cancer in the year 2010[1]. A total of 1,596,670 new cancer causes and 571,950 deaths are projected to occur in United States in 2011[2].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, in most cancers 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[3-6].

Urinary bladder cancer (UBC) ranks the fourth most common cancer in men and ninth in the women in United States, accounting for an estimated 70,980 new cases in 2009[7]. Numerous studies have demonstrated that the primary modifiable risk factors for UBC are cigarette smoking and occupational exposure to carcinogens. An updated review of the literature estimates that between 5 and 25% of all UBC cases are attribute able to workplace exposures [8]. The variance in attributable fraction suggest that, despite numerous studies investigating the association between UBC risk and occupational exposures, result have been inconsistent. To date, only few occupations and industries, such as those exposed to aromatic amines, have been unequivocally associated with increased risk of UBC.

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, lessexpensive, biodegradable, eco-friendly and widely available[9].

The development of new applications for chitosan and its derivative is mainly due to the factthat 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 to create new ones. Chitosan is modified natural, biodegradable, biocompatible, nontoxic, as well as linear nitrogenous polysaccharides, polysaccharide homo-polymer[10].

Use of natural biopolymers for diversified applications in life sciences has several advantages, such as availability from replenishable agricultural or marine food resources, biocompatibility, biodegradability, therefore leading to ecological safety and the possibility of preparing a variety of chemically modified derivatives for specific end uses. Polysaccharides, as a class of natural macromolecules, have the tendency to be extremely bioactive and are generally derived from agricultural feedstock or crustacean shell wastes. Cellulose, Starch, Pectin etc.Chitin and Chitosan next to cellulose. The application potential of chitosan is multidimensional, such as in food and nutrition, biotechnology, material science, drugs and pharmaceuticals[11].

Chitosan, composed of β-(1 - 4)-linked N-acetyl-D-glucosamine (GlcNAc unit) and deacetylatedglucosamine ($GlcNH_2$ unit) are obtained by deacetylation of chitin, a major component of exoskeleton in crustaceans and also a cell wall component of fungi. Chitosan have various biological activities including antimicrobial activity[12], antioxidant activity[13], immuno-enhancing effects[14], and antitumor activity[15]. Those activities attracted more attentions, especially the activity of antitumor. The antitumor activity of Chitosan was first reported in early 1970s[16]. This activity was suggested mainly due to its cationic property exerted by amino groups, and later it was accepted that the molecular weight also plays a major role for the antitumor activity[17]. Recently, it was proved that strong electronic charge is an important factor for anti-cancer activity of Chitosan[18]. Furthermore, some researchers found that antitumor effects of Chitosan were due to increased activity of natural killer lymphocytes as observed in Sarcoma 180-BearingMice[19].

Though the antitumor activity of Chitosan has been studied in vivo and in vitro, the molecular mechanisms of the antitumor are still unclear. In the present study reveals that comparative cytotoxic efficacy of Chitosanand cyclophosphamide against the Bladder carcinoma cells (T 24 cell line).

MATERIALS AND METHODS

Drugs and chemicals

Chitosan was obtained as a gift from M/s. apex laboratories, Chennai, MEM was purchased from Hi medic laboratories, Fetal bovine serum(FBS) was purchased from cistronlaboratories, Trypsin, methylthiazolyldiphenyl-tetrazolium bromide(MTT) and Dimethyl sulfoxdie(DMSO) were purchased from (Sisco research laboratory chemicals, Mumbai). Cyclophosphamide was procured from adyar cancer institute, Chennai, India.

Cell culture conditions

T-24 Human Bladder Cancer cell line was obtained from National centre for cell sciences Pune (NCCS), India.The cells were maintained in Minimal Essential Media supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO_2 at 37 °C.

In vitro assay for Cytotoxicity activity

The Cytotoxicity of samples on T-24 cells was determined by the MTT assay [20].The effect of the chitosan on the proliferation of T-24 was expressed as the % cell viability, using the following formula:

% cell viability = A570 of treated cells / A570 of control cells × 100%.

RESULTS

Inhibitory effect on proliferation of T24 cells

The effect of Chitosan and cyclophosphamide on the cells viability was measured by the MTT assay, which reflects the cellular reducing activity. MTT assay as shown in Table -1&Fig. 1 indicated that chitosan and cyclophosphamide inhibited the T-24 cells proliferation in a concentration and dose dependent manner.The median lethal concentration of Chitosanand cyclophosphamide was 62.5µg/ml for T24 at 48h.After 48 hours the percentage of cell viability of the chitosan and cyclophosphamide were 52.7 % and 47.1% respectively. Fig. 3.showed that different concentration of chitosan and cyclophosphamide wasinhibited cell viability on T24 cell line. The present study shows chitosan has a well pronounced cytotoxic effect on T24 cell line (52.7%) when compared to cyclophosphamide (47.1%).

[Pls insert here Figure and slide]

DISCUSSION

Kun TeShenet et al[21] demonstrated the protective effect of chitosan on the proliferation of HepG2 cells and suppress tumor growth in HepG2-bearing SCID mice. Chakraborty et al[22] demonstrated that modified chitosan had in vitro cytotoxicity against HeLa cell lines in Swiss mice. Hosseinzadeh et al[23] proved that chitosan nanoparticls had inhibition cell viability on HT-29 colon carcinoma cell line.Ming Jiang et al[24] demonstrated that chitosan derivatives inhibit cell proliferation and induce apoptosis in MCF-7 breast cancer cell line.

CONCLUSION

The present study exhibited the more prominent in vitro cytotoxic efficacy of chitosan on human bladder cancer T24 cell line when compared to cyclophosphamide.

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