CHAPTER V

5.0 SUMMARY AND CONCLUSION

Cancer is one of the leading causes of adult deaths worldwide. In India, the International Agency for Research on Cancer estimated indirectly that about 6,35,000 people died from cancer in 2008, representing about 8% of all estimated global cancer deaths and about 6% of all deaths in India. The absolute number of cancer deaths in India is projected to increase because of population growth and increasing life expectancy. Rates of cancer deaths are expected to rise, particularly, from increases in the age specific cancer risks of tobacco smoking, which increase the incidence of several types of cancer. India is a culturally diverse country, with huge regional and rural-to-urban variation in lifestyles and in age-specific adult death rates. (Ferlay et al, 2010).

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 (Yamada et al., 2010).
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 antitumour studies showed that intravesical chitosan/IL-12 is superior to both intravesical IL-12 alone and BCG, the standard of care immunotherapy for the treatment of superficial bladder cancer in a murine model (David et al., 2009).

João 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 in vitro and in vivo.

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.

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. Further, The antimicrobial effect of chitosan base pharmaceutical preparations has not yet been studied.

The objective of the present study is 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 and antimicrobial effects of chitosan invivo.

Design of the study

The study was carried out in three phases

**PHASE - I – Screening of invitro antimicrobial and antioxidant efficacy of chitosan and its pharmaceutical preparations.**

The following parameters were evaluated invitro to assess antioxidant and antimicrobial property of chitosan.

a) Film forming property of chitosan based pharmaceutical preparations

b) Antimicrobial Activity of chitosan based and chitosan non based (Fucidin) pharmaceutical preparations

c) Wound healing property of chitosan based and chitosan non based (Fucidin) pharmaceutical preparations
d) Evaluation of Free radical Scavenging and antioxidant property of chitosan

(Invitro study)

i) Scavenging ability of chitosan on 1, 1-diphenyl 1-2-picryl hydroxyl radicals (DPPH)

ii) Hydroxyl radical scavenging ability of chitosan

iii) Superoxide anion radical scavenging ability of chitosan

iv) Reducing power of chitosan

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

The following assay / analysis were carried out to assesses the antiproliferative efficacy of chitosan on T24 urinary bladder cancer cell line.

a) Inhibitory effect on proliferation of T24 cells (MTT assay)

b) Apoptosis by DNA fragmentation study

c) Cell cycle distribution analysis

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

Lipid peroxidation, enzymic antioxidants (superoxide dismutase, catalase, glutathione peroxidase and reduced glutathione) and non enzymic antioxidants (Vitamin E and Vitamin C) were analysed in bladder tissue.
a) Lipid peroxidation
b) Enzymic and non enzymic antioxidants
c) Histopathological examination

SALIENT FINDINGS OF THE STUDY

PHASE – I

a) Film forming property of chitosan based pharmaceutical preparations

The film forming properties of chitosan based and chitosan non based formulations in the various categories of cream like antibacterial cream (Fusidic acid cream, Calcium mupirocin), antifungal cream (Ketoconazole, Miconazole and terbinafine Hcl ) was carried out to support the antimicrobial property. The present study demonstrate the more pronounced film forming ability of chitosan based semi solid preparations when compared to chitosan non based (Fucidin) formulations.

b) Antimicrobial Activity of chitosan based and chitosan non based pharmaceutical preparations

The result of the present study showed the highest zone of inhibition of chitosan (CS) against staphylococcus aureus, pseudomonas aeruginosa, Bacillus spizizenii and Escherichia coli were found to be 21.42 mm, 8.89 mm, 9.02 mm and 15.33 mm respectively at the dose of 1400 µg/well. The highest zone of inhibition of Fusidic acid (FA) against staphylococcus aureus,
pseudomonas aeruginosa, Bacillus spizizenii and Escherichia coli were found to
be 20.97 mm, 8.58 mm, 9.2 mm and 10.87 respectively at the dose of 1400 µg/well.

The highest zone of inhibition of chitosan non based Fusidic acid cream
(FC) against staphylococcus aureus, pseudomonas aeruginosa, Bacillus
spizizenii and Escherichia coli were found to be 18.65 mm, 5.94 mm, 10.89 mm
and 11.99 mm respectively at the dose of 70 µg/well.

From this Fusidic acid cream alone acts against the bacteria but the
addition of the chitosan in fusidic acid cream has got enhanced antimicrobial
functions when compared with Fucidin cream.

c) Wound healing property of chitosan based and chitosan non based
pharmaceutical preparations

In the present study, control as well as the test group showed
considerable increase in rate of % wound contraction and period of epithelization.
The mean % wound contraction was found to be better in group treated with
chitosan based Fusidic acid cream as compared to the group treated with
chitosan non based Fusidic acid cream indicating that chitosan based Fusidic
acid cream is better in wound repair.

The process of epithelization was observed depending on the day on
which the echar falls, which reveals wound healing. Faster epithelization process
is indicated by decreased number of days of falling of echar. The period of epithelization of animals of treated with chitosan based Fusidic acid cream (group 2) was comparable with animals of treated with chitosan non based Fusidic acid cream (group 1).

d) Free radical Scavenging and antioxidant property of chitosan (Invitro study)

i) Scavenging ability of chitosan on 1, 1-diphenyl 1-2-picryl hydroxyl radicals (DPPH)

The present study revealed the moderate scavenging ability of chitosan when compared ascorbic acid and Butylated hydroxyl anisole.

ii) Hydroxyl radical scavenging ability of chitosan

The present study showed highest scavenging ability of chitosan on Hydroxyl radicals as compared to standard (ascorbic acid) was reported at 40.1% at 1mg/ml. The Hydroxyl radical scavenging potential of chitosan ranged from 12.2 % to 40.1at varying concentrations (0.125 to 1mg/ml) and ascorbic acid ranged from 15% to 45 % showed moderate to high scavenging abilities respectively.

iii) Superoxide anion radical scavenging ability of chitosan
The inhibitory effect of the chitosan on superoxide anion radical was ranging from 15.20% to 32.10% for the concentration between 0.125-1 mg/ml. However, the scavenging effect of Ascorbic acid (0.125- 1 mg/ml) was found to be higher than the chitosan and the range was from 19.00% to 45.00%.

iv) Reducing power of chitosan

The reducing power of chitosan ranging from 15.47% to 19% at 0.125 to 1.0 mg/ml, where as ascorbic acid ranging from 34.5% to 98.7%. In present study chitosan showed less reducing power when compared to ascorbic acid.

PHASE - II

a) Inhibitory effect on proliferation of T24 cells (MTT assay)

The present study indicated that chitosan and cyclophosphamide inhibited the T24 cells proliferation in a concentration and dose dependent manner. The median lethal concentration of Chitosan and cyclophosphamide was 62.5 µg/ml for T24 at 48h. After 48 hours. After 48 hours of incubation, the chitosan and cyclophosphamide (anti cancer drug) were compared the the percentage of cell viability (52.7 %) and (47.1 %.) respectively.

b) Apoptosis by DNA fragmentation study

The results of the present study demonstrated that chitosan induces DNA fragmentation in T24 cell line, which correlated with necrotic tumour cell death due to disturbance of cell membrane.
c) Cell cycle distribution analysis

Chitosan induced effects were detected by comparing the cell cycle profiles between the chitosan-treated and untreated cells. The present investigation demonstrated a significant decrease of cell in the Go / G1 Phase. Apoptotic peaks were observed and cell apoptotic incidence increased in a dose dependent manner after chitosan treatment.

Phase - III

a) Lipid peroxidation

The levels of TBARS in different experimental groups of animals was assessed to reveal the tissue damage in cancer cells. The animals treated with benzidine (Group II), there was a significant increase (P< 0.001), in the level of thiobarbituric acid reactive substances (TBARS) as compared to all other groups and normal control (Group I). The animals treated with chitosan in benzidine induced rats (Group III), there was significant decrease in the level of TBARS when compared to benzidine induced rats (Group II) which might be the antiproliferative property of chitosan.

b) Enzymic and non enzymic antioxidants

The enzymic antioxidants such as superoxide dismutase (SOD), catalase (CAT) glutathione peroxidase (GPx), glutathione reductase (GR) and non enzymic antioxidants such as glutathione (GSH), Vitamin E and Vitamin C were
found to be significantly reduced in group II benzidine treated animals. In animals treated with chitosan (Group III) the antioxidant enzymes levels were significantly increased ($p<0.01$) to near normal levels when compared to Group II animals.

The present study unveil the free radical scavenging and antioxidant effects of chitosan in benzidine induced bladder cancer in mice

c) Histopathological examination

The present study demonstrates the antioxidant efficacy of chitosan in benzidine induced bladder cancer in swiss albino mice. This finding is supported by the histopathological examination in different experimental group of mice.

The following conclusion can be drawn from the present investigation

**Phase - I** study demonstrated the antioxidant and antimicrobial properties of chitosan in pharmaceutical preparations. (Invitro study)

**Phase - II** revealed that antiproliferative and cytotoxic efficacy of chitosan on T24 bladder cancer cell line (Invitro)

**Phase - III** exemplify the antiperoxidative efficacy of chitosan benzidine induced bladder cancer in mice. (Invivo study)
General recommendations for the population who are prone to benzidine exposure.

1. Benzidine is rarely present in air, soil and water and used for dye in colour food.
2. All public health providers and public should be aware of toxic effects of benzidine and its preventive measure. Children should not be allowed to enter in area, where benzidine and other toxic chemicals are exposed.
3. Better prevention and treatment for benzidine used in industries should enable us the control the health effect of benzidine.
4. Daily intake of fruits and vegetables rich in antioxidants would be beneficial to control the toxic effects of various chemicals including benzidine.

6.0 SCOPE AND RECOMMENDATIONS

1. The effect of chitosan on bladder cancer in human population needs to be studied.
2. Further studies is needed to demonstrate the active principles and antioxidants from natural sources including medicinal plants for control and treat bladder cancer.