INTRODUCTION

Cancer is the second leading cause of the death among human population. It is multifactorial in origin with heterogeneous nature and is characterised by uncontrolled cell division and spread of abnormal cells causing massive aggregation to produce tumour growth. Primary cause of cancer may be due to physical, chemical or biological factors or in combination. Cancer causing factors induce progression of the disease by alteration in genetic material leading the normal cell to cancer cell which is termed as carcinogenesis.

Carcinogenesis involves initiation, promotion and progression, where initiation can occur after a single, brief exposure to a potent initiating agent but it is irreversible. The initiated cells will have defect in maturation, escapes from senescence and have altered dependence on growth factors and hormones. The promotion phase is a slow, gradual process and requires more prolonged exposure to the promoting agent. Tumour promotion is a cell proliferation phase that propagates the initiated damage and leads to the emergence of an altered clone of cells and inhibits apoptosis. Promotion occupies where the greater part of carcinogenesis process, is reversible, and can be arrested by certain anticarcinogenic agents. Tumour progression requires continued clonal proliferation of altered cells, during which a loss of growth control and an escape from host defence mechanisms become predominant phenotypic traits. Progression is accelerated by additional exposure to genotoxic agents including genetic instability and non-random sequential chromosomal aberrations and malignant conversions produce premalignant lesions. This process allows growth to progress to a clinically detectable tumour.

Proliferation and differentiation involves many genes which are mainly involved in cell cycle control, apoptosis, DNA repair, aging, immortalization, angiogenesis and metastasis. The main oncogenes involved in the tumor progression and proliferation are ras, src, MAP kinase, raf, NF-kB, Bcl-2, c-myc, cyclin D etc. and tumor suppressor genes are P53, Rb, PTEN etc.

Conventional strategies used in cancer treatment include surgery, chemotherapy, and radiation therapy hence each modalities having limitations in clinical use. Surgery is only possible for solid tumors. Ionizing radiations used in cancer therapy exert damaging effects in normal tissues and induce complex response at cellular and molecular level. When a chemotherapeutic drug enters the circulation, these agents not only kill the cancer cells but also the normal cells which result in side effects including organ toxicity and myelosuppression. The other modalities of cancer treatment with biological materials as monoclonal antibodies, cytokine therapies, gene therapy etc are still under primitive stage and some of them are not giving expected promising results. There is an urgent need to find out the cancer medicines which having anticancer properties and at the same time nontoxic to the normal cells.

The discovery of new effective and safe anticancer drugs will benefit millions suffering from this disease. Plants and plant derived products identified are successfully used in the treatment of different types of cancer. Nine of the current top 20 drugs, with annual sales of around $ 16.5 billion are derived from or based on natural products. Many natural dietary compounds in fruits and vegetables have been isolated and have demonstrated health-promoting properties. Many herbal drugs and their isolated compounds are also found to possess chemopreventive potential which enables them to intervene one or more steps in the process of carcinogenesis. The steps that are targeted for chemoprevention for inhibition of the malignant transformation of initiated cells include scavenging reactive oxygen species, altering gene expression, decreasing inflammation, suppressing proliferation, inducing differentiation, encouraging apoptosis, enhancing immunity or inhibiting angiogenesis and metastasis. The chemoprevention is also possible at the cell cycle regulatory pathways (Surh, 2003).

The adjuvant role of herbal drugs as radioprotectors and chemoprotectors is reported by several workers (Nair et al, 2001; Davis and Kuttan, 1998; Kumar and Kuttan, 2004). An ever-growing list of putative cancer chemopreventive agents, including many that are derived from medicinal products and dietary constituents. These include terpenes, alkaloids, polyphenols and their synthetic derivatives, which have been shown to trigger apoptosis in tumour cells in vivo and/or in vitro. These bioactive compounds are mostly plant secondary metabolites, and many naturally occurring pure compounds have become medicines, dietary supplements, and other useful commercial products. Important anticancer drug targets include tubulin, DNA topoisomerases I and II (topo I and topo II), cyclin dependent kinases (CDKs), growth and transcription factors, etc. These compounds include Vinca alkaloids and Camptotheca alkaloids as well as modified related compounds. The Vinca alkaloids vinblastine (A1) and vincristine (A2) are well-known anticancer drugs.

Carotenoids are naturally occurring plant pigments that are involved in light-harvesting reactions and protect plant organelles from singlet-oxygen-induced damage. The carotenoid like β-carotene, lycopene and lutein present in fruits and vegetables exert antioxidant functions such as quenching of singlet oxygen and other electronically excited molecules and reduces the progression of many degenerative diseases (Di Mascioet al., 1989. Many fruits and vegetables are rich sources of carotenoids. Lutein (3,3’-dihydroxy-β,ε-carotene) is a carotenoid, is abundantly present in nature, is commercially prepared from marigold flower (*Tagetuserecta* L)). Lutein, zeaxathin and mesozeaxanthin are the only macular pigments and due to their extended conjugated structure have been shown to have significant antioxidant potential and a protective effect against the oxidative damage to macula induced by singlet oxygen produced by ultraviolet light. Lutein and zeaxathin are important carotenoid components in the human diet and several investigators have suggestedthat elevated intake of food rich in lutein is related todecreased macular degeneration and the risk of cataracts (Stahl& Sies, 2005) . Recent studies have suggested that lutein can reduce atherosclerosis and affords cardiac protection. Lutein also reduces skin damage induced by ultraviolet rays

(Rodrigues & Shao 2004). There is an urgent need for developing an effective chemo preventive dietary compound to reduce the incidence of cancer. Lutein is non- toxic and is considered as GRAS (generally regarded as safe) by Food and Drug Administration US as a nutritional supplement. Hence in the present study we have evaluated the anticancer, chemopreventive as well as chemoprotective activities, mechanism of action and other pharmacological actions of lutein.

OBJECTIVES OF THE PRESENT STUDY

1. To determine the antioxidant activities of lutein (in vitro and in vivo)
2. To determine the antimutagenic effect of lutein using mutated strains of *Salmonella typhimurium* strains (Ames test)
3. To evaluate the inhibition of chemical carcinogenesis as well as anitumour activity of lutein
4. To study the renal protective activity of lutein on cisplatin treated mice as well as chemoprotective activity on cyclophosphamide treated mice.
5. To determine the radioprotective activity of lutein
6. To evaluate the antiinflmmatory, gastroprotective and hepatoprotective activity of lutein.

METHODS AND RESULTS

The link between free radicals and disease process has lead to considerable research with the aim to discover the nontoxic drugs that can scavenge the free radicals and thereby halt the causation and progression of the diseases. Presently carotenoid lutein was evaluated for its antioxidant potential both in vitro and in vivo. Significant antioxidant activity was found to be exhibited by lutein both in vitro and in vivo. Lutein was found to scavenge superoxide radicals, hydroxyl radicals and inhibited in vitro lipid peroxidation. Concentrations needed for IC_{50} were 21, 1.75 and 2.2 μg/mL respectively. It scavenged 2,2-diphenyl-1-picryl hydrazyl (IC_{50} 35 μg/mL) and nitric oxide radicals (IC_{50} 3.8 μg/mL) while 2,2-azobis-3-ethyl benzthiozoline-6-sulfonic acid radicals were inhibited only at higher concentration. Ferric reducing power (50%) of lutein was found to be equal 0.3μmols/mL of $FeSO_4.7H_2O$. Oral administration of lutein inhibited superoxide generation in macrophages in vivo. Oral administration of lutein in mice for 1 month significantly increased the activity of catalase, superoxide dismutase, glutathione reductase and glutathione in blood and liver while the activity of glutathione peroxidase and glutathione-S-transferase were found to be increased in the liver tissue. These studies confirmed the antioxidant potential of lutein.

Ames test is widely accepted to identify the chemicals and drugs which can produce gene mutation and has a high predictive tool for in vivo carcinogenicity (Michaud et al., 2000). Lutein was investigated for its antimutagenic activity in vitro by Ames test using *Salmonella typhimurium* strains TA 98, TA 100, TA 102 and TA 1535. Mutagens used were direct acting mutagens such as sodium azide (5μg/ plate), nitro-o- phenylendiamine (20μg/ plate), N-methyl- N'-nitro-N-nitrosoguanidine

(1µg/ plate), tobacco extract (50mg/ plate) and acetamidofluorene (20µg/ plate) which needed microsomal activation. Lutein significantly inhibited the mutagenicity produced by direct acting mutagens as well as mutagens needing activation by cytochrome P450 enzymes at very low concentration (IC_{50}<50 µg/plate). Lutein also inhibited the mutagenicity induced by tobacco extract (IC_{50}<50 µg/plate). These results confirmed that lutein is an antimutagenic agent against direct acting mutagens as well as mutagens needing metabolic activation.

Lutein was checked for anticarcinogenic activity against several carcinogens such as N-nitrosodiethylamine (NDEA), 7,12dimethylbenz[a]anthracen (DMBA) and 3- methyl cholanthrene (3-MC). Lutein could significantly reduce the altered morphological and pathological changes in the liver induced by NDEA. Biochemical analysis of serum and tissues indicated that alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) which were significantly elevated in control group and were significantly reduced in lutein treated groups. These enzymes in liver tissue which were found to be elevated in control group were significantly reduced in lutein treated groups. Glutathione level was low in control groups and it was found to be increased in treated groups. The activity of γ-glutamyl transpeptidase (GGT), a marker of cellular proliferation was found to be significantly elevated in both serum and liver in control group which was reduced by lutein administration. Inhibition of chemical carcinogenesis by lutein in 3-MC induced sarcoma in micewere also studied. Lutein was found to delay the onset of sarcoma in methyl cholanthrene induced animals and increase the lifespan of animals. Lutein also inhibited carcinogenesis at two stages produced by the application of DMBA followed by croton oil application. Lutein could significantly delay the on set of papilloma in mice. Papilloma formation was found to be significantly inhibited by the simultaneous skin application of lutein. Studies on the mechanism of action of lutein indicated that it could significantly inhibit cytochrome P 450 enzymes in vitro and in vivo in rats. Moreover lutein could enhance the detoxifying enzymes glutathione-S-transferase and UDP glucuronyl transferase in vivo. Inhibition of carcinogenesis by lutein could be due to a combined effect of its antioxidant activity along with inhibition of cytochrome P450 enzymes and inducing detoxifying enzymes.

Lutein at a concentration of 14 µg/mL was found to produce 100% cytotoxicity to Dalton's lymphoma ascites tumor cells. Moreover lutein could significantly increase the lifespan of ascites tumor bearing animals by the lutein treatment. The solid tumor development was also found to be inhibited significantly by lutein treatment. The results from these studies indicated that lutein has strong anticarcinogenic activity against chemically induced as well as inhibited transplanted tumours in animal models.

In the present study we also have evaluated the nephroprotective activity of lutein to reduce the cisplatin induced renal damage in mice. Serum urea and creatinine levels in the cisplatin induced

mice were significantly elevated compared to normal group and it was reduced by the lutein treatments ($P<0.01$)). The antioxidant enzymes in the kidney such as superoxide dismutase, catalaseactivities and level of reduced glutathione were declined and the level of malondialdehyde was elevated in the controlas well as in vehicle control groups. These enzymes were significantly increased by lutein treatment and the level of malondialdehyde declined significantly in treated groups. WBC count and bone marrow cellularity which were significantly lowered in control groups were also significantly elevated in all lutein treated groups ($P<0.001$). Study concluded that lutein could effectively protect the kidney of mice treated with cisplatin which was also supported by histopathology of kidney. Cyclophosphamide is an anticancer drug inducing myelosuppression. Myelosuprresion protective activity of lutein was evaluated in cyclophosphamide treated mice. WBC count and bone marrow cellularity were significantly lowered in control groups which were also significantly elevated in all lutein treated groups ($P<0.001$) and it confirmed the chemoprotective effect on haematopoetic system.

Lutein pre-treatment significantly reduced myelosuppression during radiation as evident from increase in WBC count, bone marrow cellularity and number of maturing monocytes in lutein treated animals when compared to radiation control animals. Antioxidant enzymes and glutathione in both liver and intestinal mucosa which were found to be decreased after irradiation and these were markedly elevated by lutein administration. Lutein showed significant anti-clastogenic activity as seen from decreased number of micronuclei formation and chromosomal aberrations in lutein pre-treated animals when compared to radiation control. Irradiation also resulted in damage to cellular DNA as evidenced by comet formation where the comet parameters like percentage of DNA in tail, tail length, tail moment of bone marrow cells in radiation control animals were found to be increased and these damages were decreased by lutein treatment. Results confirmed the radioprotective potential of lutein in irradiated animal models.

Lutein was checked for the anti-inflammatory activity against acute inflammatory agent like carrageenan and dextran as well as chronic inflammatory agent formalin induced paw oedema. The paw oedema was significantly elevated in control groups and this oedema significantly decreased by lutein treatment in a dose dependent manner in both acute and chronic inflammatory models. The findings were confirmed the antiinflmmatory activity of lutein. Gastroprotective activity of lutein was studied in ethanol induced gastric ulcer models. The ulcer index which is a measure of the severity of ulcers was found to be reduced in lutein-treated groups. Morphological and histopathological examination supported the protection oflutein during alcohol induced damage in rat stomach. Antioxidant enzymes such as catalase, super oxide dismutase and glutathione peroxidase as well as glutathione levels in gastric mucosa of lutein treated groups were found to be reduced in control groups and they were elevated in lutein treated groups. These findings suggest the potential therapeutic use of lutein as an effective antiulcer agent. Hepatoprotective activity of lutein was studied

using three models of hepatotoxins. Paracetamol, which is common antipyretic agent, is safe in therapeutic dose but in high dose causing liver damage, was used for the study as a hepatotoxin. The other two models are carbon tetrachloride as well as ethanol. Carbon tetrachloride intoxication in rats is widely used to study necrosis and steatosis of the liver. Liver, which can metabolise ethanol, shows a profound alteration in intermediary metabolism when subject to high doses or with lengthy exposure. Levels of serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase and alkaline phosphatases, which were increased in the serum by this treatment, were found to be significantly reduced by the treatment of lutein in a dose-dependent manner. The data presented in this study support the hypothesis that lutein may protect liver from various other toxic substances by effectively preventing the oxidative stress.

The thesis has been divided into 8 chapters as follows:

Chapter 1: Review of literature.

Chapter 2: Materials and methods.

Chapter 3: Antioxidant activity of lutein

Chapter 4: Antimutagenic activity of lutein

Chapter 5: Anticarcinogenic and antitumor activity of lutein and its mechanism of action

Chapter 6: Nephroprotective and chemoprotective activity of lutein

Chapter 7: Radioprotective activity of lutein

Chapter 8: Anti-inflammatory, gastroprotective and hepatoprotective activity of lutein

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