Summary & conclusion
Cancer is recognized as being a highly heterogeneous disease within individual tumors, and within and between tumor types. This heterogeneity is manifested at both genetic and phenotypic level, and primarily determines the self-progression of neoplastic disease and its response to therapy. The progressive transformation of normal cell to a highly malignant derivative or neoplasm strongly depends upon change in interactions between malignant cells and their normal neighbours. Tumor tissue grows as an abnormal analogue of the tissue of its origin, as it is influenced or governed by interplay of multiple basically normal physiologic processes and corresponding regulatory mechanisms (Lokitnov, 2004).

In spite of advances and development in the field of technology and medicine, cancer still continues to be a threat to mankind. The therapeutic modalities available ranging from conventional to most modern targeted therapies fail many times in case of cancer. Toxic side effects including multiple organ failure are the major drawback of radiation and chemotherapy. The multi drug resistance is another problem. Cancer cells are able to metastasize as they migrate from the primary tissue and hence escape from many conventional therapeutic agents. Cancer cells also are resistant to many immunological barriers in the body. As cure is not practical for several cancers in the present scenario, search for newer therapeutic agents with potential activity and without side effects are undergoing in several research centers all over the world. Plants and plant derived products identified are successfully used in the treatment of different types of cancer.

Carotenoids are naturally occurring plant pigments that are involved in light-harvesting reactions and protect plant organelles from singlet-oxygen-induced damage. Carotenoids such as 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 Mascio et al., 1989). Lutein is an important carotenoid component in the human diet and several investigators have suggested that elevated intake of food rich in lutein is related to decreased 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 activities of lutein.

In the present study we have studied the antioxidant, antimitagenic, anticancer and antitumour activities of lutein using *in vitro* and *in vivo* models. The possible mechanism of action of lutein against chemical carcinogenesis was studied. The study also extended to find out the effect of lutein in protecting mice from radiation and other chemotherapeutic drug induced damages. Apart from the chemopreventive and chemoprotective activities of lutein, we have studied other pharmacological activities such as anti-inflammatory, gastroprotective and hepatoprotective activity of lutein were also studied.

The oxidative stress can cause cellular damage and ROS oxidize critical cellular components such as membrane lipids, proteins, and DNA. These oxidative damages have been associated with diverse patho-physiological events, including cancer, atherosclerosis, diabetics, renal disease, and neuro-degeneration (Seitz & Stickel, 2006, Halliwell, 2001). 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 of lutein needed for IC$_{50}$ values were 21, 1.75 and 2.2 μg/mL respectively. Lutein 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 benzthiazoline-6-sulfonic acid radicals were inhibited only at higher concentration. Ferric reducing power (50%) of lutein was found to be equal to 0.3μmols/mL of FeSO$_4$.7H$_2$O. 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 increase 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, nitro-o-phenylendiamine, N-methyl- N’-nitro-N-nitrosoguanidine, tobacco extract and acetamidofluorene 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 ($IC_{50}<50 \mu g/plate$). Lutein also inhibited the mutagenicity induced by tobacco extract ($IC_{50}<50 \mu g/plate$). 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 NDEA, DMBA and 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 ALT, AST and 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 mice were 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 onset 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. Cisplatin has been reported to cause nephrotoxicity in patients as well as in a variety of animal species (Badary et al. 1997). 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, catalase activities and level of reduced glutathione were declined and the level of malondialdehyde was elevated in the control as well as in vehicle control groups. These enzymes were significantly increased and the level of malondialdehyde was declined significantly by lutein treatment. 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 (Dumontet et al. 2001). Myelosupression 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.

Ionizing radiation, employed in radiotherapy of various cancers, is nonselective in its action because it affects both tumor cells and normal cells. Radiation-induced biological damage in biological systems was found to be mediated through the generation of reactive oxygen species (ROS) in cells as a result of water radiolysis (Kamat et al., 2000). Total body exposure to ionizing radiation damages rapidly proliferating cells especially bone marrow cells. 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 edema. The paw edema was significantly elevated in control groups and this edema significantly decreased by lutein treatment in a dose dependent manner in both acute and chronic inflammatory models. The findings were confirmed the anti-inflammatory 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 of lutein during alcohol induced damage in rat stomach. Antioxidant enzymes such as catalase, superoxide 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.

Lutein was found to be non-toxic to rats up to a concentration of 5 gm/kg. body weight (Harikumar et al. 2008). Administration of lutein increases the concentration in serum and in tissues. Lutein is accumulated more with lipids (Viswanathan et al. 2009). As lutein is non-toxic as well as safe dietary compound, it stands as one of the prime compounds in the
chemoprevention trials in the future. The encouraging result obtained from the present study is suggesting that lutein may be developed as an effective chemotherapeutic agent. Further studies are needed to elucidate the molecular mechanisms involved to prove lutein’s efficacy as an anti-cancer agent. The present work demands further investigation to prove underlying mechanisms involved in anticancer activity of lutein.