Summary and conclusions
Spices which possess a pleasant aroma and taste, form major components of many food items, soft drinks and beverages. The compounds derived from spices and their essential oils are fascinating sources of natural products with fewer side effects (Shoeb, 2006). This property of essential oils have attracted the attention of many researchers to investigate their uses in various diseases including cancer. The rhizome of Curcuma longa L. belonging to the family Zingiberaceae is extensively and traditionally used in many Asian countries to enhance the food quality, color, flavor and antioxidant properties (Krishnaswamy, 2008; Ruby et al., 1995). Turmeric essential oil (TEO) is isolated from rhizome of Curcuma longa L by the process of steam distillation. TEO is different from oleoresin of turmeric where curcuminoids are the major compounds while ar-turmerone is the major constituent of TEO (Sacchetti et al., 2005). Some medicinal and pharmacological properties of TEO have been reported (Negi et al., 1999; Jayaprakasha et al., 2002; Sacchetti et al., 2005; Funk et al., 2010; Prakash et al., 2011). In the present study, we have evaluated the anticancer, apoptotic potential, anti-mutagenicity, radioprotective as well as chemopreventive potential of TEO from Curcuma longa. We have also evaluated toxicity profile and GC/MS analysis of TEO. To determine the possible mechanism of action, we have valuated the effect of TEO on different cytochrome P450 enzymes and phase II enzymes.

Chemical analysis of TEO was done by gas chromatography-mass spectrometry (GC/MS). TEO contained 13 compounds of which ar-turmerone (61.79%), curlone (12.48%) and curcumene (6.11%) were found to be prominent. Acute administration of TEO was done as single dose of 5 g of TEO per kg body weight and LD$_{50}$ was observed to be > 5000 mg/kg body weight. Subchronic toxicity study for thirteen weeks was done by daily oral administration of TEO at doses 0.1, 0.25 and 0.5 g/kg body weight in rats. There was no mortality, adverse clinical signs or changes in body weight; water and food consumption during acute as well as subchronic toxicity studies. Indicators of hepatic function such as aspartate aminotransferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP) were unchanged in treated animals compared to untreated animals. TEO administration for 13 weeks did not alter total cholesterol, triglycerides, markers of renal function, serum electrolyte parameters and histopathology of tissues.
TEO did not produce any mutagenicity to Salmonella typhimurium TA-98, TA-100, TA-102 and TA-1535 with or without metabolic activation. Administration of TEO to rats (1 g/kg b.wt.) for 14 days did not produce any chromosome aberration or micronuclei in rat bone marrow cells and did not produce any DNA damage as seen by comet assay confirming the non-toxicity of TEO. The results of the present study indicate that the TEO is safe in rats (NOAEL) up to an oral dose of 0.5 g/kg body weight.

Antioxidant, anti-inflammatory, anti-nociceptive and anti-ulcer properties of TEO was evaluated. TEO was found to have \textit{in vitro} antioxidant activity and IC$_{50}$ for scavenging superoxides, hydroxyl radicals, and lipid peroxidation were 135 µg/ml, 200 µg/ml and 400 µg/ml, respectively. The ferric reducing activity for 50 µg of TEO was found to be 5mM. Intraperitoneal administration of TEO was found to inhibit PMA-induced superoxide radicals elicited by macrophages. Oral administration of TEO for one month to mice significantly increased superoxide dismutase, glutathione, and glutathione reductase enzyme levels in blood and glutathione-S-transferase and superoxide dismutase enzymes in liver. TEO showed significant reduction in paw thickness in carrageenan, dextran induced acute inflammation, formalin induced chronic inflammation. The drug produced significant antinociceptive activity (p<0.001) at all doses studied. TEO inhibited ulcer by 84.7% as seen from the ulcer index. TEO administration significantly (p<0.001) enhanced antioxidant enzymes (Catalase, GSH and GR) present in gastric mucosa and prevented gastric ulcer. Histopathological examination showed that ethanol-induced lesions such as necrosis, erosion and hemorrhage of the stomach wall were significantly reduced after oral administration of essential oil.

Antimutagenic potential of TEO was assessed by using \textit{Salmonella} strains against known mutagens with and without microsomal activation. TEO showed significant antimutagenic activity (p<0.001) against direct acting mutagens such as sodium azide (NaN$_3$), 4-nitro-O-phenylenediamine (NPD) and N-methyl- N-nitro N’nitrosoguanine (MNNG). TEO was found to have significant antimutagenic effect (>90%) against mutagen needing metabolic activation such as 2-acetamidofluorene (2-AAF). The study also revealed that TEO significantly inhibited (p<0.001) the mutagenicity induced by tobacco extract in Salmonella TA 102 strain. In this study, we have indicates TEO has
strong antimutagenic potential against known chemical mutagens and tobacco with and without metabolic activation.

We have evaluated the cytotoxicity and antitumour activity of TEO. TEO was found to have significant *in vitro* cytotoxic activity against Dalton’s lymphoma ascites cells (DLA) and Ehrlich ascites carcinoma (EAC) cancer cell lines. Concentration needed for 50% cytotoxicity (IC50) was 8 μg for DLA cells and 18 μg to EAC cell lines. MTT assay indicated TEO exhibited dose-dependent decrease of cell proliferation in L929, HeLa, HepG2 cancer cell lines with IC50 values of 32 μg/ml, 42.3 μg/ml, 53.7 μg/ml respectively. Morphological changes in HepG2 cell treated with TEO were observed by florescent and phase contrast microscopy and the presence of apoptotic bodies and condensed chromatin was also seen in TEO treated HepG2 cells indicating the induction of apoptosis. Oral administration of TEO was found to significantly increase the life span (56.25%) of Dalton’s Lymphoma Ascites (DLA) induced ascites tumour bearing mice and significantly reduced (P<0.001) the solid tumours. Besides, RT-PCR analysis of the expression of apoptotic genes in TEO treated HepG2 cells showed expression of caspase and p53 genes. These genes are actively participated in intrinsic pathway of apoptosis indicating that TEO is an effective antineoplastic agent in future.

The protective effects of TEO as a chemopreventive agent against NDEA induced hepatocellular carcinoma in rats, two-stage mouse skin papilloma development induced by DMBA as initiator and croton oil as promoter and 3-methyl cholanthrene (3-MC) induced sarcoma in mice were also evaluated. Oral administration of TEO 20, 100 and 500 mg/kg body weight) restored NDEA induced alteration in liver weight, morphology and biochemical as well as histopathological changes. TEO significantly reduced the NDEA induced tumour nodule formation in the liver. Elevated hepatic parameters in the serum (ALP, ALT, and AST) as well as gamma-glutamyl transferase (γ-GT) level were decreased by TEO in a dose dependent manner. Moreover TEO could protect the animals from NDEA induced oxidative stress by restoration of antioxidant enzyme levels. DMBA and croton oil induced papilloma development in mice was found to be delayed and prevented (50% TEO) significantly by TEO application. 3-Methylcholanthrene (3-MC)
induced sarcoma development was also delayed and there was significant increase in the life span of mice treated with 3-MC after oral administration of TEO.

The mechanism of action of TEO for their chemopreventive potential was investigated by studying their effect on phase I (cytochrome p450) and phase II enzymes (glutathione-S-transferase (GST) and UDP-glucuronyl transferase) in vivo and in vitro. Phase I enzymes introduce polar group into xenobiontic compounds whereas phase II enzymes conjugate with polar groups of carcinogens to make them water soluble substances which are excreted out. TEO significantly (P<0.001) inhibited various isoforms of cytochrome p450 enzymes CYP1A2 (MROD), CYP2B 1/2 (PROD), CYP1A1 (EROD), CYP 2E1 (aniline hydroxylase), and CYP 1A, 2A, 2B, 2D and 3A (aminopyrene-N-demethylase), which are involved in the activation of carcinogens. Levels of Phase II enzymes –UDPGT and GST were found to be significantly increased by administration of turmeric essential oil in a dose dependent manner. Amelioration of NDEA, DMBA and 3-MC induced carcinogenesis by TEO could be due to its antioxidant activity and inhibition of metabolic activation of carcinogens by enhancing detoxification of the mutagenic substance in liver by inducing phase II enzymes.

Radioprotective efficacy of TEO was studied after whole body gamma irradiation. Radiation exposure (6Gy) resulted in a significant inhibition in hematological parameters (Hb and BC) and hematopoiesis (Bone marrow cellularity and α-esterase positive cells). TEO treatment in mice showed a gradual recovery and at 14th day all parameters levels were increased significantly to normal level (P<0.001). Oral administration of TEO gradually restored all these antioxidant enzyme levels (Catalase, GPx, SOD, GR and GSH) in both liver and intestinal mucosa at 3rd, 7th, and 14th days in a concentration dependent manner. TEO could significantly repair or protect gamma-radiation (3Gy) induced chromosomal damages (chromosomal aberrations, chromatid breaks, ring formation and fragmentation in bone marrow cells) as well as micronuclei formation and single and double strand breaks in DNA in mice. Moreover histopathological changes in small intestine produced by radiation were repaired by administration of TEO. These results indicate that TEO provided protection against radiation-induced damage and alterations in mice.
The effects of TEO on the growth of *Aspergillus flavus* and its production of aflatoxin were also done. Aflatoxin B1 is produced by *Aspergillus species* is the most potent naturally occurring mycotoxin/carcinogenic substance. The results showed that TEO significantly inhibited the mycelia growth (dry weight) of *Aspergillus flavus*. TLC analysis showed a significant decrease in the production of aflatoxin in TEO treated sample compared with the control. The quantification of aflatoxin using fluorospectrometer after TEO treatment, indicated that TEO inhibited the aflatoxin production by 95%. Antimutagenic activity of TEO against the aflatoxin B1 was evaluated using *S. typhimurium* showed that TEO significantly (P<0.001) inhibited the mutagenicity induced by aflatoxin B1 in a concentration dependent manner.

Toxicity study was conducted to evaluate the effects of TEO (500 mg/kg body weight) against aflatoxin B1 induced toxicity in ducklings. Ducklings are the most sensitive organism towards aflatoxin. Oral administration of aflatoxin B1 reduced the body weight of ducklings. Aflatoxin B1 produced significant changes in hematological (WBC, RBC, Hb and Platelet) and serum biochemical parameters as well as in the histology of organs which support the toxicity of aflatoxin B1. Results showed restoration of these parameters after oral administration of TEO indicating that TEO can reduce aflatoxin B1 induced damages in ducklings. Renal and liver functional parameters are also stabilized by TEO. TEO significantly decreased aflatoxin B1 induced GGT levels in liver. Aflatoxin B1 treatment changes in histo parameter such as necrosis, haemorrhage and vacuolation in various tissues were restored upon administration of TEO indicating that it can inhibit the toxicity of aflatoxin B1 in ducklings.

TEO was evaluated for its anti-carcinogenic activity against aflatoxin B1 induced hepatocellular cancers in Wistar rats. Liver is the primary target organ of metabolic action of aflatoxin and it is highly sensitive to liver as well as induces hepatic carcinoma. There was a significant restoration of aflatoxin altered serum and tissue biochemical parameters (ALT, AST, ALP and bilirubin), and GGT level indicating the protective effect of TEO aflatoxin B1. Aflatoxin B1 reduced the activities of antioxidant enzymes catalase, GST, GPx and GSH significantly which were restored by TEO. TEO
significantly reduced aflatoxin induced lipid peroxidation level in liver. Histological observations also supported the protective ability of TEO. It could be concluded that TEO could significantly inhibit liver carcinogenesis induced by aflatoxin B1 in a dose dependent manner.

In cancerous tissues free radical production reduces the ability to meet the energy demands of the cell by reducing the levels of mitochondrial TCA cycle enzymes. Administration of aflatoxin B1 significantly decreased the levels of TCA cycle enzymes such as isocitrate dehydrogenase, succinate dehydrogenase, NADH-dehydrogenase and malate dehydrogenase. TEO restored their levels near to normal. Glucose- 6-phosphatase and fructose-1-6-diphosphatase which are marker enzymes for liver microsomal activity is greatly inhibited in cancer bearing animals which revealed a progressive failure of gluconeogenesis in cancerous conditions. In TEO treated groups the levels of gluconeogenic enzymes were found to be elevated when compared to aflatoxin induced rats and were approximately nearer to untreated groups. In cancer cells, the rate of energy liberation by carbohydrate metabolism is very high for the growth and development of cancerous tissues since the hexokinase and 3 gluco-6-phospho-dehydrogenase enzymes activities also increased. The level of hexokinase and 3-gluco-6-phospho-dehydrogenase increased due to the long term administration of aflatoxin B1 was significantly reduced by the TEO administration in rats. It has been shown that aflatoxin B1 is metabolically activated by hepatic cytochrome P450 enzymes to produce a reactive intermediate, aflatoxin B1-8,9-epoxide, which consequently binds to nucleophilic sites in DNA and the major adduct 8,9-dihydro-8-(N7 guanyl)- 9-hydroxy aflatoxin B1 is formed. TEO significantly decreased the aflatoxin metabolic enzymes (Cytochrome p450) in rat’s liver and reduced both the DNA adducts and protein carbonyl formation. Hence it can be concluded that, TEO have significant protective effect against aflatoxin induced toxicity and hepatocarcinogenesis.

These results indicated that in addition to imparting flavour and antioxidant to food, turmeric essential oil also possess potential health benefits by enhancing the free radicals scavenging antioxidant enzymes formed in the body, and thereby act as nontoxic anti-inflammatory, anti-nociceptive, anti-ulcer, anti-tumor agent as well as it induces
caspase dependent pathway of apoptosis in cancer cells. TEO can act as a non-toxic agent to reduce the side effects of radiation. Moreover, regulate the level of drug metabolizing enzymes that TEO can increase carcinogen detoxification in the body and thereby act as a cancer-preventing agent.
Biological and chemopreventive potential of turmeric essential oil