Abstract
Essential oils from spices are gaining greater interest as natural antioxidants, food preservatives and additives. Essential oils contain a number of chemical constituents, and this complex combination of compounds gives its characteristic fragrance and flavor. The use of essential oils is increasing rapidly in most of the countries in food industries, cosmetics, oral care products, aromatherapy and pharmacology. Their potential to prevent or treat various types of cancers have been investigated in recent years. Turmeric, which is the rhizome of *Curcuma longa* L. (Zingiberaceae), has greater demand in most Asian countries where it is an important ingredient of food to impart aroma and flavor, besides its antioxidant property. In the same way, turmeric essential oil (TEO) from *Curcuma longa* has been reported to have several biological and pharmacological properties. In the present study, we have evaluated the toxicity profile, chemical composition, anticancer activity, anti-mutagenicity, radioprotective as well as chemopreventive potential of TEO. We have also assessed the potential of TEO against aflatoxin induced toxicity, hepatocarcinogenesis and adduct formation in animals. To determine the possible mechanism of action, we have evaluated the effect of TEO on different cytochrome P450 enzymes (CYP450), phase II enzymes and apoptotic gene expressions.

The safety of TEO was examined in a series of toxicology studies, as the first step to support its future research activities and its use among general population, using acute (5g/kg b.wt.), subchronic (0.1, 0.25 and 0.5 g/kg b.wt.) and genotoxicity in Wistar rats. There were no observed mortality, adverse changes in body weight and other biochemical parameters during the acute and subchronic toxicity studies. Oral administration of TEO 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 2 weeks did not produce any chromosome aberration or micronuclei formation 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 b.wt. for 13 weeks.
The main constituents of essential oil of turmeric were found to be ar-turmerone (61.79%), curlone (12.48%) and ar-curcumene (6.11%). TEO was found to have in vitro antioxidant activity and the observed IC50 values 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 5 mM. Intraperitoneal administration of the oil was found to inhibit PMA-induced superoxide radicals elicited by macrophages. Oral administration of TEO to mice significantly increased superoxide dismutase, glutathione, and glutathione reductase enzyme levels in blood. Similarly, liver enzymes such as glutathione-S-transferase and superoxide dismutase enzymes were also observed to be elevated. TEO showed significant reduction in paw thickness in carrageenan or dextran-induced acute inflammation and formalin-induced chronic inflammation model in mice. Moreover, the drug produced significant antinociceptive activity (P < 0.001) at all doses studied. TEO could also reduce the gastric ulcer in rat stomach as seen from the ulcer index and histopathology of the stomach. Furthermore, TEO administration significantly enhanced antioxidant enzymes such as catalase, GPx, SOD and GSH present in gastric mucosa and reduced gastric damage.

We have evaluated the cytotoxicity, anti-proliferative, antitumor and apoptotic 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) cell lines. TEO showed significant anti-proliferative activity against various cell lines such as L929, HepG2 and HeLa and found to be moderately cytotoxic to Vero cell line in a concentration dependent manner. 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 reduce the solid tumour volume in mice. TEO treated HepG2 cells showed typical morphological characteristics of apoptosis including plasma membrane blebbing, nuclear and cytoplasmic condensation and cell shrinkage. Besides, RT-PCR analysis of the expression of apoptotic genes in TEO treated HepG2 cells showed that caspase 3, caspase 9 and p53 was showing several fold increase. These
genes are actively participated in intrinsic pathway of apoptosis indicating that TEO is an effective antineoplastic agent in future.

TEO showed significant antimutagenic activity (p<0.001) against direct acting mutagens such as sodium azide (NaN₃), 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-acetamidoflourene (2-AAF). The study also revealed that TEO significantly inhibited (p<0.001) the mutagenicity induced by tobacco extract to Salmonella TA 102 strain. To evaluate the chemopreventive activity of TEO, we assessed three carcinogenic models such as NDEA induced liver carcinogenesis in rats, DMBA induced skin papilloma in mice and 3-methyl cholanthrene induced sarcoma development in mice. Oral administration of TEO significantly reduced the NDEA induced tumor nodule formation in the liver and restored the levels of hepatic marker enzymes, antioxidant enzymes and γ-GT (Gamma-glutamyltranferase). Moreover, TEO significantly delayed and prevented the skin papilloma development in DMBA and croton oil treated mice. 3-Methylcholanthrene induced sarcoma development was also delayed and there was significant increase in the life span of mice after oral administration of TEO. Besides, TEO significantly (P<0.001) inhibited various isoforms of cytochrome p450 (CYP1A1, CYP1A2, CYP2B1/2, CYP2A, CYP2B and CYP3A) enzymes as well as enhanced the elimination and detoxification of the mutagenic substance in liver by inducing phase II enzymes such as UDP-glucurononyl transferase and GST.

Radioprotective efficacy of TEO was studied after whole body gamma irradiation in mice. TEO can protect highly radiosensitive organs such as bone marrow, peripheral blood cells and intestinal epithelium from irradiation. Radiation exposure (6Gy) resulted in a significant inhibition in hematological parameters (Hb and TC) and hematopoiesis (Bone marrow cellularity and α-esterase positive cells). Oral administration of TEO in irradiated mice gradually restored the antioxidant enzyme levels (Catalase, GPx, SOD, GR and GSH) in both liver and intestinal mucosa. TEO could significantly repair or protect gamma radiation (3Gy) induced chromosomal aberrations as well as micronuclei
formation and single and double strand breaks in DNA in mice bone marrow cells. Furthermore, histopathological changes in small intestine produced by radiation were repaired by the administration of TEO.

The effects of TEO on the growth of *Aspergillus flavus* and its production of aflatoxin were also analysed. The results showed that TEO significantly inhibited the mycelia growth and aflatoxin production by 95%. TEO significantly (P<0.001) inhibited aflatoxin B1 induced mutagenicity and toxicity in ducklings in a concentration dependent manner. TEO was evaluated for its anti-carcinogenic activity against aflatoxin B1 induced hepatocellular carcinoma in Wistar rats. There was a significant restoration of aflatoxin-induced alteration in serum and tissue biochemical parameters (ALT, AST, ALP and bilirubin), and γ-GT levels indicating the protective effect of TEO against aflatoxin B1. Aflatoxin B1 reduced the activities of antioxidant enzymes such as catalase, GST, GPx and GSH significantly which were restored by TEO. Histological observations also supported the protective ability of TEO. TEO also restored aflatoxin altered mitochondrial enzymes (TCA cycle enzymes) in rats. Besides, TEO significantly decreased the aflatoxin metabolic enzymes (CYP450) in rat’s liver and significantly reduced both the DNA adducts and protein carbonyl formation. From these studies, we can conclude that the TEO has a prominent role in showing protective activity against *A. flavus* and its toxin. These results indicated that, in addition to imparting flavour and antioxidant to food, TEO also possesses non-toxic and potential health benefits. Moreover, it could regulate the level of drug metabolizing enzymes and induce apoptosis in cancer cells. Hence TEO is found to increase carcinogen detoxification in the body and thereby can act as a cancer-preventing agent or anti-neoplastic agent.

**Key words:** Turmeric essential oil; ar-turmerone; antitumor; apoptosis; anti-mutagenicity, radioprotective; chemopreventive; hepatocarcinogensis; cytochrome p450; Aflatoxin; DNA adduct.