Introduction

Cancer is a disease which involves alterations in the expression of multiple genes that confer a survival advantage and proliferative potential to somatic or germinal cells. More than 11 million people are diagnosed with cancer every year. It is estimated that there will be 16 million new cases every year by 2020 (Cho, 2007). Alterations primarily in three main classes of genes \textit{viz.}, (proto) oncogenes, tumor suppressor genes and DNA repair genes collectively contribute to the development of cancer genotype and phenotype. Cancer cells display a broad spectrum of genetic alterations that include gene rearrangements, point mutations, and gene amplifications, leading to disturbances in molecular pathways regulating cell growth, survival, and metastasis. The resistance in the natural and inherent death mechanism embedded in cells (apoptosis), coupled with dysregulation of cell proliferation events suggest that cancer is also driven by ‘epigenetic changes’ like DNA methylation and altered patterns of histone modifications, leading to alterations in chromatin condensation status.

Free radical generation is mainly unprogrammed, but unavoidable in aerobic organisms due to their reliance on oxidative processes for life. Cellular redox balance is maintained by a powerful antioxidant system that neutralizes ROS. It consists of superoxide dismutase, catalase, the glutathione system (glutathione, glutathione reductase, peroxidase and transferase), the thioredoxin system (thioredoxins, thioredoxin peroxidase and peroxiredoxins), vitamin E and C. Oxidative stress generally describes a condition in which cellular antioxidant defense mechanisms are insufficient to inactivate ROS, excessive ROS is produced, or both. It is well-documented that oxidative stress damages lipids, proteins, sugars and nucleic acid bases, which reduces cell viability and cellular functions (Therond, 2006). Evidence exists that cancer cells are under a continuous oxidative stress and that tumor-associated inflammation is an important player in the neoplastic process. Inflammation is also increasingly recognized as an important component of tumorigenesis (Karin, 2006). ROS-induced cell death seems to be distinctively controlled in normal and neoplastic cells by different pro-/anti-apoptotic factors and by antioxidant key elements.

Existing anti-cancer therapies exert deleterious effects on normal tissues, partially triggered by ROS generated, which limit the applicable dosage and their anti-tumor activity. Radiotherapy is one of the clearest examples of antineoplastic treatment whose mechanism relies primarily on ROS. Most cancer chemotherapy regimens make use of highly cytotoxic drugs that
target proliferating cell populations. A major drawback of current cancer therapeutic practices such as chemotherapy and radiation therapy is bone marrow suppression resulting in cytopoenia and subsequent suppression of humoral and cellular responses (Devasagayam and Sainis, 2002). The non-discriminatory use of these drugs leads to severe side effects in normal cells with high proliferative index, thus leading to drug resistance and limiting the effective dose of anticancer drug that can be administered. Overcoming these side-effects, without altering the efficacy of the therapy, is a research priority. There is a tremendous historical legacy in folklore use of plant preparations in medicine. Development of naturally derived anticancer drugs is therefore crucial and isolation of novel compounds has become an important part of cancer research.

Scientific studies of plants used in ethnomedicine have led to the discovery of many valuable drugs, which act as anticarcinogens and reduce side effects of chemo and radiation therapy. Many plant derived compounds can modulate diverse biochemical processes involved in carcinogenesis. These include inhibition of CYP450-dependent activation of carcinogens, induction of phase II detoxification enzymes, trapping reactive carcinogenic species, blockade of tumor cell proliferation and induction of apoptosis in malignant cells (Hail et al., 2008).

Spices have been of particular interest in basic science research in relation to chronic disease risk as they contain many phytochemicals, including flavonoids, tannins, phenolic acids, and terpenes, that may be alleviate these diseases. The pharmaceutical properties of aromatic plants are partially attributed to essential oils. Essential oils are recognized for their bioactivity as antibacterial, antiviral, anti fungal, antioxidant and antidiabetic agents. Their application as natural skin penetration enhancers for transdermal drug delivery is known. The therapeutic properties of essential oils have been utilized in aroma and massage therapy. The diverse therapeutic potential of essential oils has drawn the attention of researchers to test them for anticancer activity, taking advantage of the fact that their mechanism of action is dissimilar to that of classic cytotoxic chemotherapeutic agents (Rajesh, et al, 2003).

Ginger, the rhizome of *Zingiber officinalis*, which is one of the most widely used species of the ginger family, is a common condiment for various foods and beverages. Ginger has been traditionally used from time immemorial for varied human ailments in different parts of the globe, to aid digestion and treat stomach upset, diarrhoea, and nausea. Phenolic compounds such as gingerols and related compounds, which are responsible for the pungent taste of ginger, have
been a major focus of research. It has been reported that ginger and its extracts show pharmacological activities including antiinflammation, (Mascolo et al., 1989; Penna et al., 2003), antiemesis (Sharma and Gupta, 1998), analgesic effect (Young et al., 2005), cytotoxic (Unnikrishnan and Kuttan, 1988), anti-tumor (Katiyar et al., 1996) and anti-oxidant (Masuda et al., 2004) activities.

The essential oil extracted by steam distillation from the rhizomes of ginger is also reported to possess pharmacological properties. It was also reported to have antibacterial, antiviral, and antioxidant activity (Gurdip et al., 2008, Koch et al., 2008). It could suppress chronic adjuvant arthritis induced in both paw and knee of Sprague-Dawley rats (Sharma et al., 1984). Essential oil of ginger shows immunomodulatory activities (Zhou et al., 2008). In the present study, we have evaluated the pharmacological, anticancer, radioprotective, chemopreventive properties and mechanism of action of GEO.

**OBJECTIVES OF THE PRESENT STUDY**

1. Determination of the components, toxicity, and bioavailability of ginger essential oil
2. Investigation of the antioxidant, anti-inflammatory, antinociceptive and antiulcer properties of ginger essential oil
3. To check the mutagenic and antimutagenic effect of ginger essential oil against both direct acting mutagens and indirect acting mutagens by Ames test.
4. To study the cytotoxic and antitumor activities of ginger essential oil.
5. Evaluation of the anticarcinogenic potential of ginger essential oil against chemical carcinogens and its possible mechanism of action.
6. Analysis of radioprotective potential of ginger essential oil
7. To find out ameliorative activity of ginger essential oil against aflatoxin induced toxicity

**METHODS AND RESULTS**

Ginger essential oil (GEO) isolated from *Zingiber officinale*, was provided by Kancore Ingredients Limited., Angamali, Kerala, India. GEO was dissolved in paraffin oil for all *in vivo* studies. For *in vitro* studies, the GEO was dissolved in hexane (100 mg/10ml) and 10 µl of
Triton X 100 (Sigma-Aldrich) was added and further evaporated to dryness and finally made up to 10 ml with distilled water.

The chemical composition of GEO was analyzed by GC-MS. The main constituents of essential oil of ginger were zingiberene (31%), ar-curcumene (15.4%) and α-sesquiphellandrene (14.02%). Other compounds include bisabolene (13.80%) and sabinene (8.27%).

Toxicity of ginger essential oil was evaluated by acute administration of GEO up to 5 g/kg b.wt and subchronic administration for 13 weeks at doses of 100, 250 and 500 mg/kg body weight for 90 days in Wistar rats. There was no mortality, no change in body weight and food consumption as well as organ weight during the study period. Administration of ginger oil did not produce any change in hematological parameters, hepatic, renal functions, serum electrolytes and in histopathology of selected organs. Initial studies indicated the presence of zingiberene in the serum after oral dosing. Study confirmed that ginger oil is not toxic in rats following oral administrations up to a dose level of 500 mg/kg body weight.

The antioxidant, anti-inflammatory and antinociceptive potential was studied. Antioxidant activity of GEO was assayed both in vitro and in vivo. Ginger oil scavenged superoxide, DPPH, hydroxyl radicals and inhibited tissue lipid peroxidation in vitro. Intraperitoneal administration of ginger oil was found to inhibit PMA induced superoxide radicals elicited by macrophages. Oral administration of ginger oil for one month, significantly increased superoxide dismutase, glutathione and glutathione reductase enzyme levels in blood of mice and glutathione-S-transferase, glutathione peroxidase and superoxide dismutase enzymes in liver. Ginger oil produced significant reduction in acute inflammation produced by carrageenan and dextran and formalin induced chronic inflammation (p<0.001). Antinociceptive activity was measured using acetic acid induced writhing test. GEO exhibited significant reduction in acetic acid induced writhing movements (p<0.001). These studies revealed that ginger oil possess antioxidant activity as well as significant anti-inflammatory and antinociceptive property. Essential oil isolated from ginger was also analyzed for anti-ulcer activity against ethanol-induced ulcers in male Wistar rats. The dosage of essential oil administered were 100, 500, and 1000 mg/kg b. wt. Parameters such as ulcer index, histopathology, levels of antioxidant enzymes such as GPx, SOD, catalase as well as glutathione levels were measured to assess the degree of protection. GEO administration reduced the ulcer index significantly (85% in 1000 mg/kg b.wt GEO treated rats) and restored the reduced GPx, catalase, SOD and glutathione levels during
ethanol-induced gastric ulceration. Histopathological examination showed ethanol treatment induced lesions such as necrosis, erosion and hemorrhage of the stomach wall was significantly reduced by essential oil treatment indicating the anti-ulcer action of GEO.

Cytotoxic and antitumor activity of GEO was studied. GEO showed potent *in vitro* cytotoxic activity against DLA and EAC cell lines. IC$_{50}$ value for DLA cell line was 11 μg/ml and for EAC cell lines 18 μg/ml. The IC$_{50}$ of GEO was found to be 41 μg/ml against the L929 cancer cell line. The antitumor activity of GEO was determined using DLA cell line induced solid tumor and EAC cell lines induced ascites tumor model in mice and its comparison with standard anticancer drug cyclophosphamide. The treatment with GEO (500 mg/kg and 1000 mg/kg body weight) significantly reduced the solid tumor development by 54.4% and 62.4% respectively. The life span of the animals was increased up to 50% in 1000 mg/kg b.wt GEO treated animals. This indicates that GEO has significant anticancer effect *in vitro* and *in vivo*.

Use of antimutagens and anticarcinogens in everyday diet is the most effective way for preventing human cancer and genetic disorders. Antimutagenic activity of GEO was tested in *Salmonella typhimurium* strains- TA98, TA100, TA102 and TA1535 by Ames test. GEO which was found to be non-mutagenic up to a concentration of 3 mg/plate was also assessed for antimutagenic potential against direct acting chemical mutagens such as sodium azide, 4-nitro-o-phenylenediamine, N-methyl-N’-nitro-N-nitrosoguanidine, tobacco extract and mutagen needing microsomal activation, 2-acetamidoflourene. GEO was found to inhibit the mutagenicity induced by the agents (p<0.001) in a concentration dependent manner.

Chemopreventive potential of ginger essential oil (GEO) was assessed using three different carcinogenic models. This included N-nitrosodiethylamine (NDEA) induced hepatocellular carcinoma model in Wistar rats, 3-methylchloanthrene (3-MC) induced fibrosarcoma model and skin papillomagenesis using 7, 12 - dimethylbenz (a) anthracene (DMBA) as initiator and croton oil as promoter in Swiss albino mice. Simultaneous administration of GEO (20, 100, 500 mg/kg b.wt) with NDEA suppressed the nodule incidence induced by NDEA, improved the hepatocellular architecture and significantly inhibited NDEA induced elevation of serum biochemical indices (serum glutamate oxaloacetic transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), and bilirubin as well as γ-glutamyl transpeptidase (GGT) levels in a dose dependent manner. The oxidative stress induced in the liver by NDEA as indicated by reduced GPx, GSH
and GST levels were also restored to normal levels by GEO. Treatment with GEO (20, 100, 500 mg/kg b.wt) significantly reduced the tumor incidence and prolonged the survival rate of mice harboring 3-MC induced fibrosarcoma. Skin papillomagenesis studies using 7, 12 - dimethylbenz (a) anthracene (DMBA) as initiator and croton oil as promoter demonstrated a significant increase in latency period, and decrease in papilloma formation in the case of mice topically applied with 50, 25 and 10% GEO. These results are suggestive of a potential chemopreventive activity of GEO.

Cytochrome P450 are an important group of phase I xenobiotic metabolizing enzymes. They also play a major role in mutagenesis and carcinogenesis as most of the carcinogens are metabolized to their active form by these enzymes. Effect of GEO to modulate the action of carcinogen metabolizing enzymes was investigated by studying their effect on various isoforms of cytochrome P450 enzymes. Significant inhibition of CYP1A1, CYP1A2 and CYP2B1/2, aniline hydroxylase (an indicator of CYP 2E1 activity) and aminopyrine demethylase (indicator of CYP 1A, 2A, 2B, 2D and 3A activity) was shown by GEO both in vitro and in vivo. Ginger oil also significantly increased the levels of phase II enzymes- Glutathione-S-Transferase and UDP-glucuronyl transferase in vivo indicating the use of GEO as a potential chemopreventive agent.

The radioprotective effect of ginger essential oil (GEO) on mortality, body weight alterations, hematological parameters, chromosomal damages and antioxidant activity were studied in irradiated mice. Regression analysis of survival data yielded LD50/30 as 7.12 and 10.14 Gy for control (irradiation alone) and experimental (GEO-treated irradiated) mice, respectively, and produced a dose reduction factor (DRF) of 1.42. In mice exposed to whole-body gamma-irradiation (6 Gy), GEO pre-treatment at 100 and 500 mg/kg b.wt (orally) significantly ameliorated the radiation decreased hematological and immunological parameters. Radiation also reduced the intestinal tissue antioxidant enzyme levels such as superoxide dismutase, catalase, glutathione peroxidase and glutathione which were restored following administration of GEO. Tissue architecture of small intestine which was damaged following irradiation was improved upon administration of GEO. Anticlastogenic effect of GEO was studied by micronuclei assay, chromosomal aberration and alkaline gel electrophoresis assay. GEO significantly decreased the formation of micronuclei, inhibited the formation of chromosomal aberrations and protected cellular DNA damage in bone marrow cells as revealed by comet assay. These results are indicative of the use of GEO as a potential radioprotective compound.
Aflatoxins are groups of fungal toxins that are produced by different species of fungi such as *Aspergillus flavus*. They are known to cause severe contamination to feeds and foodstuffs. Ginger essential oil was screened for its potential inhibitory activity on the growth and aflatoxin production of *Aspergillus flavus*. The inhibition of fungal growth was evaluated by determining the dry weight of mycelium. Aflatoxin production was analyzed using TLC plates and quantified by fluorospectrometer. Ginger essential oil inhibited the growth and aflatoxin production by *Aspergillus flavus* effectively. 20 µl of ginger essential oil gave maximum inhibitory effect on growth and aflatoxin production. The results point out the use of ginger essential oil in food preservation to control the aflatoxin contamination in many foods and represented a possible supplement in food industry. We have looked into the effect of ginger essential oil on aflatoxin B<sub>2</sub> induced mutagenicity on *Salmonella typhimurium* strains TA 98 and TA 100. Results indicated that ginger essential oil has a significant antimutagenic activity against aflatoxin B<sub>2</sub> at 500µg/plate.

A 2-week experiment was conducted in ducklings to investigate the ability of GEO (500 mg/kg b.wt) to counteract the toxic effects of aflatoxin B<sub>1</sub> (100 µg/kg body weight of AFB<sub>1</sub>) on body weight, relative organ weights, hematology and serum biochemistry. Consumption of the diet containing aflatoxin lowered the body weight of ducklings which was increased upon feeding with GEO. Ducklings had also lower liver, spleen, thymus and bursa of fabricius weights and increased weight of kidneys when fed with AFB<sub>1</sub> which were improved when fed with GEO. The relative weight of brain and pancreas were not affected by feeding with AFB<sub>1</sub> in the diet. Moreover, damaged histopathological architecture of the affected organs was restored upon administration of GEO. There was also a significant decrease in hemoglobin, total WBC count and platelet levels by aflatoxin treatment. Supplementation of 500 mg/kg b.wt GEO in the diet increased the levels of all the hematological parameters in the blood. Ducks fed with aflatoxin mixed diet had increased activities of SGOT, SGPT, ALP, GGT, serum creatinine and blood urea nitrogen. Feeding with GEO decreased the activities of all these biochemical parameters.

Aflatoxins are potent hepatotoxic and hepatocarcinogenic agents. This hepatotoxicity is thought to be mediated by their ability to generate reactive oxygen species and cause peroxidative damage. In the present investigation we assessed the ability of GEO (100 and 500 mg/kg b.wt) on aflatoxin B<sub>1</sub> (AFB<sub>1</sub>)-induced hepatotoxicity in a rat model. Hepatocellular carcinoma was induced in Wistar rats by oral administration thrice weekly for 20 weeks in single
doses of 25µg/kg body weight (total dose of 1.5mg/kg body weight). The results showed a significant increase in SGOT, SGPT, ALP, GGT and total bilirubin levels. Significant decrease in antioxidants activities of SOD, catalase, GPx, GSH and GST in the liver, as well as an increase in lipid peroxidation were also observed. GEO at both doses showed a significant reversal of altered biochemical, and antioxidant parameters showing that GEO possess anticarcinogenic activity. Histological observation confirmed the induction of tumor in aflatoxin alone fed animals and complete regression of tumor in GEO treated animals.

The modulation of glucose metabolizing enzymes activities play a vital role in the depletion of energy metabolism and leads to inhibition of cancer growth. In the present study, the effect of GEO on glucose metabolizing enzymes of aflatoxin B1 induced hepatocellular carcinoma was studied. A significant increase in the activities of key glycolytic enzymes i.e. hexokinase, and glucose-6-phosphate dehydrogenase, and decrease in gluconeogenic enzymes such as glucose-6-phosphatase and fructose-1,6-diphosphatase were observed in the liver and of aflatoxin treated animals which were restored upon administration of GEO. The activities of TCA cycle enzymes i.e., isocitrate dehydrogenase, succinate dehydrogenase, malate dehydrogenase, and NADH dehydrogenase were decreased in AFB1 induced hepatocellular carcinoma bearing animals. Administration of GEO enhanced the activities of TCA cycle enzymes. These findings suggest that GEO plays an important role in the modulation of key enzymes involved in glucose metabolism of hepatocellular carcinoma bearing animals.

These studies indicated that GEO has significant effect in the prevention of carcinogenesis. It has also found to possess significant antioxidant, anti-inflammatory, antimitogenic and antiulcer properties. It is clear from these studies that ginger essential oil may be considered as potential natural antioxidant and can be formulated as a part of daily supplements or additives to prevent oxidative stress that contributes to many degenerative diseases.

The thesis has been divided into 8 chapters as follows:
Chapter 1: Review of literature.
Chapter 2: Materials and methods.
Chapter 3: Antioxidant, anti-inflammatory, antinociceptive and antiulcer activity of ginger essential oil