Chapter 6

Antimutagenic and anticarcinogenic activities of ginger essential oil and its possible mechanism of action
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6.1. Introduction

Cancer is a growing health problem around the world. According to a recent report by the WHO, there are now more than 10 million cases of cancer reported per year worldwide. The fact that about 7 million people die from various types of cancer every year, makes this disease responsible for 12.5% of deaths worldwide, raising an overwhelming demand to develop new, more potent and effective anticancer, as well as chemopreventing agents. Cancer chemoprevention applies natural or synthetic chemical compounds to inhibit or reverse carcinogenesis and to suppress the development of cancer from premalignant lesions. In this context, dietary sources are a potential alternative source of safer chemicals which are not only anticancerous but are also antioxidants, anti-inflammatory, antimutagenic and of other physiological benefits.

Mutations occurring in somatic cells due to innate metabolic defects play a major role in the development of cancer and other chronic degenerative diseases (De Flora et al., 1996). Prevention of cancer and other mutation related diseases by favoring the intake of different naturally occurring dietary components from plants can provide potential protection against environmental mutagens and carcinogens. The identification of such potential antimutagenic and anticarcinogenic agents in food is essential for the benefit of human health.

Hepatocellular carcinoma (HCC), the most prevalent form of liver cancer, ranks third among all the cancer related mortalities. HCC is seldom detected at early stages and once detected treatment faces a poor prognosis and serious side effects in most cases. N-nitrosodiethylamine (NDEA), a well known inducer of HCC in animal species and humans, has been found to exist as contaminants in a wide variety of food products such as cheese, cured meat and fish, tobacco smoke, certain alcoholic beverages, agricultural chemicals and pharmaceuticals (Pradeep et al., 2010). The metabolism of nitrosamines is through Cytochrome P450 enzymes catalyzed α-hydroxylation, generating unstable metabolites that will alkylate the DNA at the site of activation (Ribeiro, 2000). NDEA induced hepatocellular carcinoma in rats is considered to be the most commonly used experimental model to study hepatocarcinogenesis (Loeppky, 1999).

Fibrosarcoma is a tumor of mesenchymal cell origin that is composed of malignant fibroblasts derived from fibrous connective tissue (Stout, 1948). Polycyclic
aromatic hydrocarbons are mutagenic and carcinogenic substances which are found widely as contaminants in the environment especially in soil, water, air and sediments (Fraumeni et al., 1993). 3-Methyl cholanthrene (3-MC), a polycyclic aromatic hydrocarbon, is known to induce fibrosarcomas and is widely used to induce chemical carcinogenesis.

The incidence of skin cancer is increasing and fast becoming one of the most common cancers to be diagnosed worldwide (Diepgen 2002). Chemically induced skin carcinogenesis model using initiator 7, 12 - dimethylbenz (a) anthracene (DMBA) followed by repeated applications of croton oil as promoter is useful in screening possible chemopreventive agents as the model displays a preneoplastic condition during carcinogenesis in the form of papillomas. Many genotoxic carcinogens require metabolic activation to an electrophilic form that can react with DNA. This process commonly occurs in the liver. Poly aromatic hydrocarbons, which become metabolically active by the action of cytochrome P450 enzymes produce reactive electrophiles leading to cellular toxicity, mutagenicity and carcinogenicity (Archer, 1989, Vitaglione et al., 2004).

Essential oils, formed as secondary metabolites in many spices, are used as major components in many food items, soft drinks, confectionery and beverages. Assessment of antimutagenic and anticarcinogenic potential of spices essential oils is becoming important due to their increasing consumption in pharmaceutical, agronomic and food industries, as food preservers and additives and as natural remedies (Sacchetti et al., 2005). Ginger essential oil (GEO) extracted by steam distillation is reported to possess various pharmacological properties such as antioxidant, antinociceptive, antimicrobial and antifungal activities (Vendruscolo et al., 2006; Singh et al., 2008).

There are no reported studies on the effect of GEO in cancer prevention and treatment. The present study was conducted to evaluate the antimutagenic properties of GEO against direct and indirect acting mutagens, chemopreventive efficacy of GEO on N-nitrosodiethylamine induced hepatocellular carcinoma, 3-methyl cholanthrene induced fibrosarcoma and DMBA –croton oil induced papilloma formation. We also studied the modulation of phase I and phase II carcinogen metabolizing enzymes after oral administration of GEO in Wistar rats.
6.2. Materials and methods

6.2.1. Ginger essential oil (GEO)

The essential oil was dissolved in dimethyl sulfoxide (DMSO) for *in vitro* studies. For *in vivo* studies GEO was dissolved in paraffin oil.

6.2.2. Bacterial strains

Histidine requiring *Salmonella typhimurium* strains TA 98, TA 100 and TA 1535 were supplied by Gene Bank & MTCC, Chandigarh, India. TA 98 was used to test for frame shift mutations while TA 1535 and TA 100 were used to test for base-pair substitutions. TA 102 strain was a gift from Dr. Padma Ambalam, Sourashtra University, Gujarat, India. They were incubated in nutrient broth for 12 hrs and frozen permanents were prepared by dispensing in small vials and stored in -70°C in presence of DMSO (0.9%). Fresh cultures for each experiment were prepared by inoculating a small inoculam of frozen permanents in 5 ml of nutrient broth and incubating for 12 h at 37°C to a density of 1-2 x 10⁹ colony forming units (CFU). The genotype of the tester strain was analyzed for their genetic integrity and spontaneous mutation rate before an experiment was done. The following tests were done for a complete strain check.

- **Histidine dependence (his):** A loopful of culture (TA 98, TA 100, TA 1535 and TA 102) was streaked across a glucose minimal (GM) agar plate supplemented with an excess of biotin. All the *Salmonella* strains are histidine dependent and no growth was observed on the plates.

- **Biotin dependence (bio):** A loopful of culture (TA 98, TA 100, TA 1535 and TA 102) was streaked across a glucose minimal (GM) agar plate supplemented with an excess of histidine. No growth was observed on the plate except for strain TA 102 which was biotin independent.

- **Biotin and histidine dependence (bio, his):** A loopful of culture (TA 98, TA 100, TA 1535 and TA 102) was streaked across a glucose minimal (GM) agar plate supplemented with an excess of biotin and histidine. Growth was observed on the plate for all the strains.

- **rfa marker:** A loopful of culture (TA 98, TA 100, TA 1535 and TA 102) was streaked across nutrient agar plate supplemented with an excess of
biotin and histidine. Sterile filter paper disc were placed containing 10 µl of sterile 0.1% crystal violet solution. All Salmonella strains showed a zone of growth inhibition surrounding the disk.

- **uvr B deletion:** A loopful of culture of R- factor strains (TA 98, TA 100, and TA 102) was streaked across nutrient agar plates. Non R-factor strain (TA 1535) was streaked across a separate plate. A piece of cardboard was placed over the uncovered plate so that half of each bacterial streak is covered. Irradiate the plate with 15W germicidal lamp at a distance of 33 cm. Non R factor strains are irradiated for 6 sec and R factor strains are irradiated for 8 sec. incubated the irradiated plates at 37°C for 24 hrs. Only the strain with uvrB deletion (TA 102) was found to grow on the irradiated side of the plate.

- **Presence of plasmid pKM 101 (ampicillin resistance):** A loopful of culture (TA 98, TA 100, TA 1535 and TA 102) was streaked across a glucose minimal (GM) agar plate supplemented with an excess of biotin, histidine and 24 µg/ml ampicillin. Sterile 6-mm filter paper disk containing 10 µg/ml ampicillin were placed on GM agar plates. Growth was observed in all the strains.

- **Presence of plasmid pAQ1 (Tetracycline resistance):** A loopful of TA 102 overnight culture was streaked across GM agar plate supplemented with excess of histidine and biotin, and 2 µg/ml tetracycline. Sterile 6 mm filter paper disks containing 2 µg/ml tetracycline were placed on GM agar plates. Growth was observed only in strain TA 102 which is the only strain that carries plasmid pAQ1.

- **Spontaneous mutant frequency:** Plate incorporation assay procedure without the inclusion of solvent was done to determine the spontaneous mutant frequency (negative control) of each tester strain. The spontaneous control values were within the historical range obtained in the laboratory.
6.2.3. Preparation of mutagens

All the mutagens, MNNG (1 μg/plate), 2-aminofluorene (20 μg/plate), sodium azide (2.5 μg/plate) were dissolved in water, except NPD (20 μg/plate) which was dissolved in DMSO.

6.2.4. Preparation of tobacco extract

100 g of tobacco were cut into small pieces and boiled in 500 ml of distilled water. This was evaporated to dryness and concentration used was 50 mg/plate. Earlier studies have reported that 50 mg of tobacco extract/plate produces maximum mutagenic response of Salmonella tester strain TA 102 (Rajeshkumar and Kuttan, 2000).

6.2.5. Antimutagenic assay

6.2.5.1. Antimutagenic assay of GEO using direct acting mutagens

Antimutagenic assay of GEO was performed by standard plate incorporation test for direct acting mutagens according to Maron and Ames (1983). GEO (50 μg, 100 μg and 1 mg/plate) was added to 2 ml of top agar containing 0.5 mM Histidine/Biotin, strains of S. typhimurium (0.1 ml having 1-2x10^9 cells/ml) and different direct acting mutagens at concentrations mentioned below. The mixture was poured into minimal agar plates and incubated at 37 °C for 48 hrs. After incubation, the number of revertant colonies was counted using a colony counter. The plates with mutagen alone acted as positive control and plates without test sample and mutagen were considered as negative controls or spontaneous revertants. DMSO (0.1 ml) which was used to dissolve GEO acted as vehicle control. The samples were tested against sodium azide (2.5 μg/plate) in strains TA 100 and TA 1535; NPD (20 μg/plate) in TA 100, TA 98 and TA 1535; MNNG (1 μg/plate) in TA 100 and TA 1535; tobacco extract (50 mg/plate) in TA 102. Each sample was assayed using triplicate plates and expressed as mean ± SD.

6.2.5.2. Antimutagenic assay of GEO against mutagen needing activation

Rat liver microsomal enzyme was used for metabolic activation of mutagen in vitro (Ames et al; 1973). Microsomal P450 enzymes were induced in two male Wistar rats (140 g) rat liver by intraperitoneal administration of phenobarbitone (60 mg/kg b.wt).
dissolved in water for 4 days. On the fifth day S9 fraction from the liver was aseptically prepared by the method of Garner et al. (1972). The effect of GEO on indirect acting mutagen, 2-acetamidofluorene (20 μg/plate), was done using *Salmonella* tester strains TA 98 and TA 100. Different concentrations of GEO (50 μg, 100 μg and 1 mg/plate), 0.1 ml bacteria (1-2x10⁹ cells/ml), activation mixture (0.5 ml) and mutagen (2-AAF) were incubated for 45 min at 37 °C. This mixture was mixed with 2 ml of top agar, and poured into minimal agar plates and incubated for 48 h at 37°C. The number of revertant colonies were counted using colony counter.

Inhibition of mutagenicity was expressed as percentage decrease of reverse mutations which was determined by the following formula:

\[
\text{Inhibition (\% of mutagenicity) = } \frac{[(R_1-SR)-(R_2-SR)]}{(R_1-SR)} \times 100
\]

where \( R_1 \) is the number of revertants induced by mutagen alone, \( R_2 \) is the number of revertants in the presence of mutagen and GEO and \( SR \) is the spontaneous revertants (Maron and Ames, 1983).

### 6.2.6. Determination of the effect of ginger essential oil on NDEA-induced hepatocarcinogenesis

Male Wistar rats were divided into six groups (8 animals/group).

- **Group I:** Normal
- **Group II:** NDEA alone
- **Group III:** NDEA + paraffin oil (Vehicle control)
- **Group III:** NDEA + ginger essential oil (20 mg/kg b.wt.)
- **Group IV:** NDEA + ginger essential oil (100 mg/kg b.wt.)
- **Group V:** NDEA + ginger essential oil (500 mg/kg b.wt.)

Group 2-6 received 0.02% NDEA in distilled water, (0.5 μl/animal/dose) by oral gavage, six days a week for 20 weeks. This dosage was found to induce liver tumors in rats. (Rajeshkumar and Kuttan, 2000). Animals were observed up to 29 weeks. At the end of the 29 weeks animals were sacrificed by administering diethylether anesthesia. Blood
samples were collected by heart puncture and serum was separated by centrifugation, and subjected to biochemical analysis.

6.2.6.1. Biochemical assays in serum and liver

The levels of glutamate pyruvate transaminase (GPT) and glutamate oxaloacetate transaminase (GOT) (Giusti et al., 1969), alkaline phosphatase (ALP) (Kind and King, 1954), and total bilirubin (Malloy and Evelyn, 1937), which are markers of liver injury, as well as gamma glutamyl transpeptidase (GGT) levels (Schumann et al., 2002) were assayed in serum using commercially available kits (Span Diagnostics, Gujarat, India).

A 25% homogenate of the liver tissue was prepared in Tris-HCl buffer (0.1 M, pH 7.4), centrifuged at 1000 rpm for 10 minutes at 4°C to remove the cell debris. The clear supernatant was used for biochemical analyses. Glutathione-S-transferase activity was determined by its ability to conjugate glutathione with 1-chloro-2,4-dinitrobenzene (Habig, 1974). Tissue levels of reduced glutathione were determined by reaction with 5, 5-dithiobis-2-nitrobenzoic acid (Moron et al., 1979). The activity of glutathione peroxidase (GPx) was determined in liver homogenate according to the method of Hafeman et al., 1974. Protein was analyzed by the method of Lowry et al., 1951.

6.2.6.2. Liver morphology and relative liver weight

Livers were excised, washed in ice cold saline (0.9%), blotted to dryness and examined for appearance of tumor nodules. Number of animals developing tumors by NDEA treatment was noted in each group and expressed as tumor incidence. Weight of livers was noted and relative liver weight was expressed as liver weight/100 g body weight.

6.2.6.3. Histopathological evaluation

A portion of the excised liver was fixed in 10% buffered formalin and stained with hematoxylin-eosin for histopathological evaluation.
6.2.7. Determination of the effect of ginger essential oil on 3-methylcholanthrene induced sarcoma

Female Swiss albino mice (20 ± 3 g, 15/group) were used for the study. The animals were divided into five groups.

Group I: 3-MC alone (Control)
Group II: 3-MC + paraffin oil (Vehicle control)
Group III: 3-MC + ginger essential oil (20 mg/kg b.wt.)
Group IV: 3-MC + ginger essential oil (100 mg/kg b.wt.)
Group V: 3-MC + ginger essential oil (500 mg/kg b.wt.)

Hair was shaven from the dorsal side of all the animals and single dose of 3-methylcholanthrene (200 µg/0.1 ml/mouse) was injected subcutaneously. Oral administration of GEO was started 24 hr before 3-methylcholanthrene injection and continued for 20 weeks, 6 days a week. The animals were observed for the onset of sarcoma as well as for their survival up to 195 days.

6.2.8. Determination of the effect of ginger essential oil on two stage skin papillomogenesis study

Female Swiss albino mice (6-8 weeks old) and weighing around 23-25g were divided into the eight groups (8 animals/group). The hair on the dorsal region of the animals was shaven 3 days before the commencement of the experiment and only those animals which did not show regrowth of hair were chosen for the studies.

Group 1: DMBA alone
Group 2: DMBA and croton oil (control)
Group 3: DMBA + croton oil + paraffin oil (vehicle control)
Group 4: DMBA + croton oil + GEO (50%)
Group 5: DMBA + croton oil + GEO (25%)
Group 6: DMBA + croton oil + GEO (10%)
Group 7: DMBA + GEO
Group 8: croton oil alone

Mice in groups 1-6, were initiated with a single topical application of DMBA (470 nmol in 200 µl acetone/animal) and after one week 1% croton oil was applied.
Ginger essential oil dissolved in paraffin oil was applied 30 min after croton oil application. As for group 8, only croton oil was applied during the promotion period. Croton oil and GEO were applied topically, twice a week for 6 weeks during promotion period and animals were observed for 20 weeks. Latency period of papilloma formation, number of mice with papilloma and number of papilloma produced/mice were observed and measured at weekly intervals. Only papillomas that persisted for more than one week with diameter greater than 1 mm were taken into consideration for data analysis.

6.2.9. Determination of the effect of ginger essential oil on carcino
gen metabolizing enzymes

6.2.9.1. Inhibition of different isoforms of microsomal cytochrome P450 enzymes by GEO in vitro

Six male Wistar rats (150-180 g) were administered with phenobarbitone for 4 days (60 mg/kg b. wt., intra peritoneally, once daily) and sacrificed 24 h after the last dose of phenobarbitone. The livers of all the rats were excised quickly, washed thoroughly in ice-cold saline and kept at -70 °C. Liver homogenate (25%) was prepared in cold phosphate buffer (pH 7.4, 0.1 M) and centrifuged at 14000 g for 20 min in Remi refrigerated centrifuge (REMI Laboratory Equipments, Mumbai, India). The separated supernatant was further centrifuged at 105000 g for 1 h in a Sorvall ultracentrifuge -RC 5C 000561, Triad Scientific Inc, USA) to separate microsomes. The microsomes were washed and resuspended in cold phosphate buffer (pH 7.4, 0.1 M) and used to determine the activities of various cytochrome P450 enzymes as given below.

The effect of GEO on the dealkylation of methoxy resorufin (MR) by 7-
methoxyresorufin-O-demethylase (MROD) representing CYPIA2, pentoxy resorufin (PR) by 7-pentoxyresorufin–O-depentylate (PROD) representing CYP2B1/2 and ethoxy resorufin (ER) by 7-ethoxyresorufin-O-deethylase (EROD) representing CYP1A1 were studied according to Pohl and Fouts, 1980 and Nerukar et al., 1993.

Assay mixtures containing sodium phosphate buffer (0.1 M, pH 7.4), 6.25 mM MgSO₄, 60 μM EDTA, 5 μM (ER, MR or PR), 100 μg microsomal protein, and various concentrations of essential oil (50, 100, 200 μg) in a final volume of 1 ml were preincubated for 5 min at 37 °C. After the preincubation period, NADPH (100 μM) was
added and incubated for 5 min at 37 °C. The reaction was stopped by the addition of 2 ml of chilled methanol. The precipitated protein was centrifuged and supernatant was used for the estimation of enzyme activity by measuring resorufin formed using a fluorescent spectrophotometer (Nanodrop ND-3300, Thermo Scientific, USA) at the excitation wavelength of 550 nm and the emission wavelength of 585 nm using resorufin as standard. Blanks were set without addition of NADPH.

The percentage inhibition = \( \frac{C-T}{C} \times 100 \)

where

‘C’ is the optical density of control without GEO,

‘T’ is the optical density with GEO.

Inhibition of aniline hydroxylase activity (an indicator of CYP 2E1 activity) and aminopyrene-N-demethylase activity (an indicator of CYP 1A, 2A, 2B, 2D and 3A activity) was measured by the method of Mazel (1971).

6.2.9.2. Inhibition of phase I enzymes by GEO in vivo

Female Wistar rats (120-150 g) were allocated in six groups comprising six animals per group.

Group I: Normal (without any treatment)

Group II: Phenobarbitone alone

Group III: Vehicle control (paraffin oil)

Group IV: GEO (250 mg/kg b.wt)

Group V: GEO (500 mg/kg b.wt)

Group VI: GEO (1000 mg/kg b.wt)

GEO was administered orally once daily for 15 days. Administration of phenobarbitone to group 2-6 was started on day 12 and continued to the 15th day (60 mg/kg b.wt, i.p., once daily). Rats were sacrificed on the 16th day and liver microsomes from normal, phenobarbitone alone, vehicle control and GEO treated animals were prepared and activities of EROD, MROD and PROD determined as given above. Calibration curves were constructed by determining the fluorescence of known amounts of the authentic resorufin. Concentration of protein in each sample was measured by the
method of Lowry et al., (1951). Results were expressed as pmols of resorufin formed /min/mg protein for *in vivo* studies.

### 6.2.9.3. Effect of GEO on phase II enzymes in vivo

Female Wistar rats (120-150 g) were allocated into five groups comprising six animals per group.

- **Group I**: Normal,
- **Group II**: Vehicle control (paraffin oil),
- **Group III**: GEO (250 mg/kg b.wt)
- **Group IV**: GEO (500 mg/kg b.wt)
- **Group V**: GEO (1000 mg/kg b.wt)

Groups III, IV and V received GEO at a dose of 250, 500 and 1000 mg/kg b.wt respectively, orally once daily for 15 days. Animals were sacrificed on the 16th day and liver homogenates prepared and used for the analysis of phase II enzymes. UDP-glucuronyl transferase activity was determined by the method of Isselbacher et al., (1962) and modified by Hollman and Touster (1962). Glutathione-S-transferase was measured by the method of Habig et al., (1974).

### 6.3. Results

#### 6.3.1. Antimutagenic activity of GEO

##### 6.3.1.1. Antimutagenic activity of GEO against direct acting mutagens

GEO was found to significantly inhibit the mutagenicity induced by different direct acting mutagens. Activity was dependent upon the *Salmonella* strain used and the nature of mutagenic substance. In all the cases, inhibition was found to be concentration dependent.

At the highest concentration used (1 mg/plate), GEO produced inhibitions of 64.7% (TA 100), 65.4% (TA 102) and 54.9% (TA 1535) to mutagenicity induced by sodium azide (Fig 6.1 A). Significant inhibitory effects of 62.4% (TA 100) and 90.3% (TA 98) were also shown against NPD induced mutagenicity at 1 mg/plate (Fig. 6.1 B). GEO was also found to inhibit mutagenicity induced by MNNG by 91.5% in TA 100 and 84.2% in TA 1535 (Fig. 6.1 C). GEO inhibited the mutagenicity induced by tobacco to
TA 102. Percentage inhibition increased with an increase in concentration, to a maximum of 77.6% at 1 mg/plate (Fig. 6.2 A).

6.3.1.2. **Antimutagenic activity of GEO against indirect acting mutagens**

Mutagenicity produced by 2-AAF after their activation with S9 fractions was found to be significantly inhibited by GEO. At concentration of 1 mg/plate, ginger essential oil gave an inhibition of 94% and 80.9% in TA 98 and TA 100 respectively (Fig. 6.2 B).

6.3.2. **Effect of GEO administration on NDEA induced hepatocarcinogenesis**

6.3.2.1. **Effect of GEO on relative liver weight and nodule incidence**

Livers of rats in NDEA treated group (Fig. 6.3 C) showed liver enlargement with macroscopic nodules compared to normal liver (Fig. 6.3 A). The livers of vehicle control treated animals also showed the same morphological features as the NDEA control group. The nodule incidence was 100% at 29 weeks for both control and vehicle control groups whereas in the case of rats treated with 500 mg/kg b.wt GEO, the nodule incidence was only 25%. The surface of liver appeared normal in GEO treated animals and there was a significant reduction in detectable lesions and nodules (Fig. 6.3 E).

A significant increase was observed in relative liver weight of NDEA treated groups when compared to normal rats. The treated groups receiving a dosage of 20, 100 and 500 mg/kg b.wt GEO showed a dose dependent reduction in the liver weight (Table 6.1).

6.3.2.2. **Effect of ginger essential oil on serum and liver biochemical parameters**

A significant increase was seen in the levels of all the serum hepatic markers (GOT, GPT, ALP), and total bilirubin in NDEA alone treated rats as well as in vehicle treated animals (Table 6.2). The animals treated with GEO showed a significant dose dependent decrease in the levels of GOT, GPT, ALP, and bilirubin (p<0.001). The activity of serum γ-GT, a marker of cellular proliferation was also found to be elevated in NDEA treated animals. Administration of GEO significantly lowered the elevated levels of γ-GT (p<0.001).
The effect of GEO on oxidative stress lowering markers GSH, GPx and GST of control, vehicle control and experimental group of rats is shown in Table 6.3. NDEA significantly reduced the GSH content in control and vehicle control group. Administration of GEO at 20, 100 and 500 mg/kg b.wt increased the levels of GSH by 64.7, 72.3 and 80.7% respectively compared to the vehicle treated groups. The levels of GPx and GST enzymes in the liver of NDEA and vehicle control group were also lowered in comparison with the normal group. GEO administration at doses 20, 100 and 500 mg/kg b.wt increased the levels of GPx by 15.3, 59.3 and 73.5% respectively. Similarly, GST levels were increased by 62.7, 91.9 and 94.3% respectively in animals treated with 20, 100 and 500 mg/kg b.wt GEO.

6.3.2.3. Histopathology analysis

Histopathology of the liver in all groups is given in Figure 6.1 B, D and F. Figure 6.1 B depicts normal liver and hepatocyte architecture. The hepatic architecture in NDEA treated group showed necrosis, haemorrhage, and inflammatory cell infiltrate. The nuclei were prominent and occupied most of the cells. Hepatocytes appeared as pleomorphic large polyhedral cells, many of them showing vacuolated cytoplasm and had vesicular nuclei. The histopathology analysis in GEO treated group (500 mg/kg b.wt) showed normal hepatocellular architecture which was almost similar to normal rat liver histology.

6.3.3. Effect of ginger essential oil on 3-methylcholanthrene induced fibrosarcoma

GEO was found to significantly inhibit the fibrosarcoma produced by 3-MC in a dose dependent manner. By the end of 15 weeks, all the animals in the control group developed sarcoma whereas only 46, 33 and 23% of animals developed sarcoma in 20, 100 and 500 mg/kg b.wt. GEO treated animals (Fig. 6.4). There was also significant increase in the survival period of mice administered with ginger essential oil (Fig. 6.5). Animals treated with 3-MC alone began dying of tumor burden 9 weeks after carcinogen treatment and all the animals died by the end of 19 weeks. Animals administered with 500 mg/ kg b.wt GEO began dying only at 13th week and in the case of 20 and 100 mg/kg b.wt GEO treated animals in the 11th week. Animals administered with ginger essential
oil showed significant increase in survival at the concentration of 20, 100 and 500 mg/kg b.wt. being 33%, 47%, 60% respectively at the end of 19 weeks (Table 6.4).

6.3.4. Effect of ginger essential oil on two-stage skin papilloma study

Papillomas began to appear on the skin from 6th week onwards in the case of carcinogen control and vehicle control treated groups during the promotion period (Fig. 6.6). Papilloma formation was significantly delayed to the tenth week in the case of GEO (50%) treated animals whereas the onset of papilloma was delayed to the eighth week in group 5 and 6 (25% and 10% GEO treated). There was no papilloma formation in groups 1, 7 and 8.

All the animals in the DMBA -croton oil treated and vehicle control group developed papillomas during the 20 weeks of study period, and the average number of papillomas formed was seven/animal in both these groups. Only 50% of animals developed papillomas in GEO (50%) treated group whereas in 25% and 10% GEO treated animals 75% animals developed papilloma. However, number of papillomas formed was only one/animal in these groups compared with that of control groups (Fig. 6.7).

6.3.5. Effect of GEO on inhibition on carcinogen metabolizing enzymes

6.3.5.1. Inhibition of cytochrome P450 enzymes by GEO in vitro

GEO was found to significantly inhibit the various isoforms of cytochrome P450 enzymes in a concentration dependent manner. Concentration needed for 50% inhibition of CYT P450 enzymes was 30, 57.5 and 40 μg for EROD, PROD and MROD respectively and 55 μg for aniline hydroxylase and 37.5 μg for aminopyrene-N-demethylase. The results indicated that GEO could significantly inhibit cytochrome P450 enzymes in vitro (Fig. 6.8 A, B).

6.3.5.2. Effect of GEO on phase I enzymes in Wistar rats

Oral administration of GEO inhibited the CYT P450 enzymes in vivo. The levels of CYT P450 enzymes were found to increase significantly following phenobarbitone administration. Phenobarbitone which is a potent inducer of phase I enzymes. Significant
inhibition in the *in vivo* levels of hepatic enzymes EROD, MROD and PROD was observed after oral administration of GEO (Table 6.5). These results indicate that GEO can significantly inhibit the activity of phase I enzymes which is needed for the activation of carcinogens.

**6.3.5.3. Effect of GEO on Phase II enzymes**

Induction of phase II enzymes is an essential property of potent chemopreventive agents as they indicate increased detoxification of carcinogens. UDP-glucuronyl transferase enzyme levels were found to be significantly increased following administration of GEO. There was also a profound increase in the activity of GST enzymes as shown in Table 6.6.

**6.4. Discussion**

Diet is known to be an important factor for determining cancer risk along with other lifestyle parameters including cigarette smoking, alcohol intake, as well as infection and disease (Sugimura et al., 1994). Essential oils are gaining greater consumer interest as natural antioxidants and food preservative agents because of their safer food status compared to synthetic food additives (Reische et al., 1998). Ginger essential oil possesses applications in pharmaceutical, food, beverage, cosmetic industry and in aromatherapy. The essential oil isolated from ginger is also known for its pharmaceutical applications. However, no study has been undertaken to evaluate the antimutagenic and chemopreventive activities of GEO and its effects in the regulation of phase I and phase II carcinogen metabolizing enzymes in animals.

The essential oil significantly inhibited the mutagenicity produced by all the direct acting mutagens, sodium azide, NPD, MNNG and tobacco and also inhibited the mutagenicity induced by 2-AAF in a dose dependent manner. The significant antimutagenic activity exhibited by GEO against direct acting mutagens suggests that it may directly protect DNA damage produced by mutagens. Mutagenicity of aromatic amine 2-AAF is dependent on metabolic activation (Wagner et al., 1994) by CYT P4501A2 and N-acetyltransferase. Inhibition of these enzymes could lead to the inhibition of mutagenicity of 2-AAF.
Studies to determine the antimutagenic activity indicated that the GEO significantly inhibited the mutagenicity produced by all the direct acting mutagens, sodium azide, NPD, MNNG and tobacco and also inhibited the activation of 2-AAF by rat liver S9 fraction in a dose dependent manner. Direct acting mutagens such as sodium azide and NPD acts by inhibiting cytochrome oxidase enzymes (Duncan and Mackler, 1966). Sodium azide also induces base pair substitutions whereas NPD acts by inducing frame shift mutations in DNA (Koch et al., 1994). MNNG acts by binding adding alkyl groups to the O6 of guanine and O4 of thymine during DNA replication (Beranek et al., 1980). Tobacco smoke is also known to contain around 20 carcinogens which are involved in inducing lung cancer in humans (Stephen, 1999). Significant antimutagenic activity exhibited by GEO against direct acting mutagens suggests that GEO may directly protect DNA damage produced by mutagen. Mutagenicity of aromatic amine 2-AAF is dependent on metabolic activation (Wagner et al., 1994) by CYT P450IA2 and N-acetyltransferase. Inhibition of enzymes (peroxidases and acetyltransferases) which is required for the activation of 2-AAF could lead to the repression of mutagenicity of 2-AAF (Plewa et al., 1991). Antimutagenic components in GEO inhibit the mutagenicity of 2-AAF by inhibiting the enzymes needed for activation of 2-AAF.

Chemoprevention of cancer involves the usage of natural or synthetic compounds to reverse or retard carcinogenesis and prevent its development to invasive cancer (Hong and Lippman, 1995). The use of plant based natural products and compounds are gaining the interest of researchers who are on the lookout of potential chemopreventive compounds. In the current study, the chemopreventive potential of ginger essential oil was studied using three different cancer models. Administration of GEO in NDEA treated animals indicated significant reduction of tumor incidence in the animals. The activities of ALT, AST, ALP and bilirubin in serum are generally accepted as indices of liver injury (Shaarawy et al., 2009). An elevation in ALT and AST concentration in serum in NDEA treated animals is attributed to the release of these enzymes from the cytoplasm into blood circulation following liver cellular damage (Karbownik et al., 2001). ALP is a membrane bound enzyme and its increase in serum may be due to alteration in membrane permeability, biliary cirrhosis, fatty liver conditions or liver tumor (Rajesh et al., 2012). Serum GGT level is considered to be a biomarker of
preneoplastic lesion (Zhang et al., 2012). An increase in GGT along with increase in serum ALP is a sure indication of hepatic cellular carcinoma. Therefore the increase of these enzymes following NDEA treatment can be correlated with hepatocarcinogenicity and development of precancerous lesions in advanced stages of liver carcinoma. Recoulement of these enzymes to near normal levels indicates the inhibitory activity of ginger essential oil against NDEA induced hepatocellular carcinoma.

Serum bilirubin serves as an index for the assessment of hepatic function and an abnormal increase in its level indicates hepatobiliary disease, obstruction of excretory ducts of the liver or severe disturbance in hepatocellular function (Martin and Friedman et al., 1998). An increase of bilirubin also indicates the inability of liver to take up and process bilirubin into bile. The significant reduction in the level of serum bilirubin after treatment with GEO indicates the protective activity of ginger essential oil.

NDEA has been known to cause the generation of reactive oxygen species (ROS) resulting in oxidative stress (Pracheta et al., 2011). NDEA is metabolized in liver and high amount of toxic intermediates are produced which may alter the antioxidant defense mechanisms present in the liver. Enhanced free radicals play an important role in tumor induction and inhibit the antioxidant and non enzymatic antioxidant activity in liver as shown by the decrease in the levels of GPx, GST and GSH in NDEA treated animals. Glutathione is a non enzymatic antioxidant which plays an important role in the protection of tissues by direct interaction of –SH group with ROS (Janssen et al., 1993). GST is involved in the detoxification of both endogenous and exogenous compounds. GST enzyme levels were also depleted which may be due to the excessive utilization of this enzyme in scavenging the free radicals (Sivaramakrishnan et al., 2008). Similarly, levels of GPx enzyme which is a constituent of GSH redox cycle was also found to be decreased during NDEA administration. The reduction in the activity of GPx on NDEA administration may be due to decrease in the availability of substrate (GSH) and also because of alterations in their protein structure by ROS (Angus et al., 1999). However, the levels of these enzymes were increased upon administration with GEO. This indicates that ginger essential oil possesses potent antioxidant activity and is benefit may be due to its effectiveness in scavenging free radicals. Previous work conducted in our laboratory has proven the ability of GEO to scavenge DPPH radicals, inhibit hydroxyl radicals, lipid
peroxidation, *in vitro* and oral administration increased the levels of SOD, catalase, GSH, GPx, GST, and GR enzymes in mice *in vivo* (Jeena et al., 2013a).

GEO significantly reduced the tumor incidence and enhanced the survival rate of mice in 3-methylcholanthrene injected mice. The tumor incidence was delayed in the treatment group when compared with 3-MC injected mice. Results indicate a significant inhibition of 3-MC, a poly aromatic hydrocarbon, induced fibrosarcoma by ginger essential oil.

It is widely believed that inhibition of tumor promotion is a better strategy in cancer chemoprevention than inhibition of tumor initiation because initiation is a short irreversible event whereas promotion is a long cumulative process that is reversible during the initial stage (Digiovanni, 1992). The present study demonstrates that the onset of papilloma formation, number of animals which developed papilloma and number of skin papillomas initiated with 7, 12 dimethylbenz (a) anthracene and promoted by croton oil were decreased by treatment with GEO in Swiss albino mice. This again indicates the potent chemopreventive potential of GEO.

Several mechanisms have been proposed for the action of antimutagens and anticarcinogens; however, one of the main mechanisms includes the inhibition of enzyme systems such as the cytochrome-P450-dependent bioactivation of the various mutagens (De Flora et al., 1992). Cytochrome P450 forms an important group of phase I xenobiotic metabolizing enzymes. They also play a major role in carcinogenesis as most of the carcinogens are metabolized to the active carcinogenic form by these enzymes.

The present study shows that administration of ginger essential oil can modulate the activities of both phase I and phase II enzymes. Phenobarbitone has been found to activate CYP 450 enzymes in the rat liver and the activation was found to be non-influenced by the gender of the animals. *In vitro* studies showed that GEO could inhibit hepatic CYP1A1, 1A2 & 2B. Oral administration of GEO also supported the *in vitro* results. CYP1A1, 1A2 & 2B subfamily metabolizes the polyaromatic hydrocarbons such as those found in charbroiled foods and cigarette smoke. CYP 2B indicated by aminopyrine-N- demethylase plays an important role in metabolism of xenobiotics. Aniline hydroxylase indicates CYP 2E1 activity, an isoform which is inducible by
ethanol, and involved in the bioactivation of variety of low molecular weight carcinogens such as benzene, styrene, nitrosamines, etc (Kalra, 2007).

GEO also significantly increased the levels of phase II enzymes. Phase II enzymes are involved in the detoxification pathways of carcinogenic compounds. Glucuronidation is a major inactivating pathway for a huge variety of exogenous and endogenous molecules. Glutathione conjugation with the help of Glutathione-S-transferase enzyme helps to detoxify and eliminate heavy metals, toxins and poly aromatic hydrocarbons. The results of the present study indicate that GEO can modulate both carcinogen activating and detoxifying pathways, and can thus act as an antimutagenic and a potential chemopreventive agent. Previous studies have also reported that ginger oil has a significant effect in increasing the level of GST enzymes (Banerjee et al., 1994). Ginger oil has also shown ability to suppress DNA adduct formation (Hashim et al., 1994).

The non-discriminatory use of highly cytotoxic drugs against proliferating cell populations leads to severe side effects in normal cells, thus limiting the effective dose of anticancer drug that can be administered (Edris, 2007). Attention is now being drawn to natural products which possess anticarcinogenic activity and lesser side effects. Earlier studies have showed that oral administration of up to 500 mg/kg b.wt. GEO is safe (NOEAL) in Wistar rats (Jeena et al., 2011). The results of the present study indicate the potential use of ginger essential oil as a chemopreventive agent. The chemopreventive potential of GEO may be due to its antioxidant activity, inhibition of carcinogen metabolism as well as induction of carcinogen detoxification.
Table 6.1: Effect of GEO on liver weights and nodule incidence during NDEA treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>Liver weight (g)/100g body weight</th>
<th>Nodule incidence /rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>3.34±0.46</td>
<td>0/8</td>
</tr>
<tr>
<td>Control (NDEA)</td>
<td>6.17±0.91</td>
<td>8/8</td>
</tr>
<tr>
<td>Vehicle control (Paraffin oil)</td>
<td>6.41±1.52</td>
<td>8/8</td>
</tr>
<tr>
<td>20 mg/kg b.wt. GEO</td>
<td>4.13±0.58***</td>
<td>3/8</td>
</tr>
<tr>
<td>100 mg/kg. wt. GEO</td>
<td>3.92±0.27***</td>
<td>3/8</td>
</tr>
<tr>
<td>500 mg/kg b.wt. GEO</td>
<td>3.41±0.37***</td>
<td>2/8</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D (n=8), ***p<0.001 compared to vehicle control group.
Table 6.2: Effect of GEO on serum bilirubin, ALP, SGPT, SGOT and γ-GT during NDEA induced hepatocarcinogenesis

<table>
<thead>
<tr>
<th>Group</th>
<th>Bilirubin (mg/100ml)</th>
<th>ALP (KA Units)</th>
<th>SGOT (IU/L)</th>
<th>SGPT (IU/L)</th>
<th>γ-GT (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.54±0.1</td>
<td>23.89±1.9</td>
<td>98.88±17.6</td>
<td>61.58±11.7</td>
<td>12.71±3.10</td>
</tr>
<tr>
<td>Control (NDEA)</td>
<td>1.44±0.2</td>
<td>139.83±2.9</td>
<td>188.35±37.0</td>
<td>104.80±12.9</td>
<td>118.8±15.2</td>
</tr>
<tr>
<td>Vehicle control</td>
<td>1.45±0.1</td>
<td>139.57±8.2</td>
<td>164.43±50.3</td>
<td>102.43±15.4</td>
<td>108.0±13.9</td>
</tr>
<tr>
<td>20 mg/kg b.wt. GEO</td>
<td>0.78±0.3***</td>
<td>131.03±11.4*</td>
<td>151.53±38.8</td>
<td>77.14±15.6**</td>
<td>58.32±11.7***</td>
</tr>
<tr>
<td>100 mg/kg b.wt. GEO</td>
<td>0.68±0.1***</td>
<td>106.70±4.7***</td>
<td>133.91±31.9</td>
<td>72.48±18.0***</td>
<td>23.49±3.3***</td>
</tr>
<tr>
<td>500 mg/kg b.wt. GEO</td>
<td>0.60±0.2***</td>
<td>98.60±2.4***</td>
<td>102.59±32.8</td>
<td>63.67±13.5***</td>
<td>14.92±2.8***</td>
</tr>
</tbody>
</table>

GEO: Ginger essential oil; Vehicle control: Paraffin oil; *** p<0.001; ** p<0.01; * p<0.05; ns: non-significant compared to vehicle control group
Table 6.3: Effect of GEO on oxidative stress markers during NDEA induced liver tumors

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH&lt;sup&gt;a&lt;/sup&gt;</th>
<th>GST&lt;sup&gt;b&lt;/sup&gt;</th>
<th>GPx&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>29.49±4.7</td>
<td>22.04±3.75</td>
<td>345.13±32.4</td>
</tr>
<tr>
<td>NDEA alone</td>
<td>12.55±1.76</td>
<td>10.32±2.13</td>
<td>187.32±27.71</td>
</tr>
<tr>
<td>Vehicle control</td>
<td>13.37±2.22</td>
<td>9.78±1.94</td>
<td>173.42±46.95</td>
</tr>
<tr>
<td>20 mg/kg b.wt GEO</td>
<td>22.02±0.65**</td>
<td>15.91±1.10**</td>
<td>200.26±14.36**</td>
</tr>
<tr>
<td>100 mg/kg b.wt GEO</td>
<td>23.03±2.21***</td>
<td>18.77±6.04***</td>
<td>276.41±23.36***</td>
</tr>
<tr>
<td>500 mg/kg b. wt GEO</td>
<td>24.16±4.55***</td>
<td>19.01±7.07***</td>
<td>300.89±45.62***</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D (n=8). ***p<0.001, **p<0.01 compared to vehicle control group.<sup>a</sup>(nmol/ml/ mg protein), <sup>b</sup>(nmol/mg protein), <sup>c</sup>(U/mg protein)
Table 6.4: Effect of ginger essential oil (GEO) on the onset of 3-methyl cholanthrene induced sarcoma in mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>65</th>
<th>90</th>
<th>120</th>
<th>150</th>
<th>180</th>
<th>195</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4/15</td>
<td>12/15</td>
<td>15/15</td>
<td>15/15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle control</td>
<td>5/15</td>
<td>8/15</td>
<td>14/15</td>
<td>15/15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 mg/kg b.wt GEO</td>
<td>6/15</td>
<td>8/15</td>
<td>11/15</td>
<td>13/15</td>
<td>14/15</td>
<td>14/15</td>
</tr>
<tr>
<td>100 mg/kg b.wt GEO</td>
<td>4/15</td>
<td>6/15</td>
<td>10/15</td>
<td>12/15</td>
<td>12/15</td>
<td>12/15</td>
</tr>
<tr>
<td>500 mg/kg b.wt GEO</td>
<td>3/15</td>
<td>6/15</td>
<td>8/15</td>
<td>10/15</td>
<td>11/15</td>
<td>11/15</td>
</tr>
</tbody>
</table>

Each group consists of 15 animals.
Table 6.5: Effect of administration of ginger essential oil on phenobarbitone induced P450 enzymes activity *in vivo*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>7-ethoxyresorufin O-deethylase*</th>
<th>7-methoxyresorufin O-demethylase*</th>
<th>7-pent oxyresorufin O-depenty lase *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>1.3±0.2</td>
<td>0.9±0.3</td>
<td>0.27± 0.4</td>
</tr>
<tr>
<td>Phenobarbitone (Control)</td>
<td>7.9±1.2</td>
<td>7.7±2.2</td>
<td>11.0±3.1</td>
</tr>
<tr>
<td>Phenobarbitone + (Vehicle control)</td>
<td>7.3±2.0</td>
<td>8.2±1.8</td>
<td>10.9±0.9</td>
</tr>
<tr>
<td>Phenobarbitone +1000 mg/kg b.wt</td>
<td>1.0±1.5a</td>
<td>0.3±0.3a</td>
<td>0.3±0.04a</td>
</tr>
<tr>
<td>Phenobarbitone + 500 mg/kg b.wt</td>
<td>1.1±1.5a</td>
<td>0.6±0.4a</td>
<td>0.3±0.2a</td>
</tr>
<tr>
<td>Phenobarbitone + 250 mg/kg b.wt</td>
<td>1.63±2.1a</td>
<td>1.2±1.1a</td>
<td>0.4±0.4a</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D (n=6). a Represents a significant difference at p<0.001 level compared with the vehicle control. Unit:* (nmol/min/mg protein).
### Table 6.6: Effect of ginger essential oil on phase II enzymes *in vivo*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>UDP-Glucuronyl transferase (nmol/min/mg protein)</th>
<th>Glutathione –S-Transferase (nmol of CDNB conjugate formed/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>31.5 ± 4.4</td>
<td>46.1 ± 6.0</td>
</tr>
<tr>
<td>Vehicle control</td>
<td>29.8 ± 5.6</td>
<td>46.6 ± 11.2</td>
</tr>
<tr>
<td>1000 mg/kg b.wt. GEO</td>
<td>41.1 ± 1.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>104.5 ± 1.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>500 mg/kg b.wt. GEO</td>
<td>37.0 ± 1.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>74.6 ± 1.5&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>250 mg/kg b.wt. GEO</td>
<td>34.7 ± 1.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>64.1 ± 1.9&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D (n=6). <sup>a</sup>Represents a significant difference at level p<0.05 compared with the vehicle control. <sup>b</sup>Represents a significant difference at level p<0.01 compared with the vehicle control. <sup>c</sup>Represents a significant difference at level p<0.001 compared with the vehicle control.
Fig 6.1. Antimutagenic activity of ginger essential oil on mutagenicity induced by direct acting mutagens

(A) Antimutagenic activity of GEO on mutagenicity induced by sodium azide

(B) Antimutagenic activity of GEO on mutagenicity induced by NPD

(C) Antimutagenic activity of GEO on mutagenicity induced by MNNG
Fig 6.2. (A) Antimutagenic activity of GEO on the mutagenicity induced by tobacco extract to TA 102

Fig 6.2. (B) Antimutagenic activity of GEO on mutagenicity induced by 2-AAF
Fig 6.3. Morphology and histopathology of liver of animals treated with NDEA and ginger essential oil (GEO)

A, Morphology of normal liver
B, Histopathology of normal liver
C, Liver treated with NDEA alone
D, Histopathology of NDEA treated animal
E, NDEA+GEO treated animal (500 mg/kg b.wt)
F, Histopathology of NDEA+GEO treated animal (500 mg/kg b.wt)
Fig 6.4. Effect of ginger essential oil (GEO) on 3-methylcholanthrene (3-MC) induced sarcoma development in Swiss albino mice

Single dose of 3-MC treated mouse showing well developed sarcoma

3-MC+ 500 mg/kg b.wt GEO treated mouse showing reduced sarcoma development
Fig 6.5. Effect of ginger essential oil on survival rate of 3-MC induced fibrosarcoma bearing mice

Fig 6.6. Effect of ginger essential oil on the onset of papilloma formation in mice initiated by DMBA and promoted by croton oil
Fig 6.7. Effect of GEO on papilloma formation after topical application of DMBA as initiator followed by croton oil as promoter for 6 weeks

DMBA+1% croton oil alone treated animal showing papilloma formation

DMBA+1% croton oil+ GEO (50%) for 6 weeks, treated animal showing reduced papilloma formation
Fig 6.8 Effect of GEO on inhibition of Cytochrome P450 enzymes *in vitro*

(A) Effect of GEO on the inhibition Cytochrome P450 enzymes- CYP1A1 (EROD), CYP1A2 (MROD) and CYP2B1/2 (PROD) *in vitro*

![Graph showing inhibition percentage vs. concentration of ginger essential oil](image)

(B) Effect of GEO on the inhibition of aminopyrene-N-demethylase (CYP 1A, 2A, 2B, 2D and 3A) and Aniline hydroxylase (CYP 2E1) activity *in vitro*

![Graph showing inhibition percentage vs. concentration of ginger essential oil](image)