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Original Article

# ANTITUMOR AND CYTOTOXIC ACTIVITY OF GINGER ESSENTIAL OIL (*ZINGIBER OFFICINALE* ROSCOE)

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**ABSTRACT**

**Objective:** To evaluate the cytotoxicty and antitumor activity of ginger essential oil (GEO).

**Methods:** Cytotoxicity towards Dalton's Lymphoma Ascites (DLA) and Ehrlich Ascites Carcinoma (EAC) cell lines were evaluated by trypan blue exclusion method. *In vitro* cytotoxicity of GEO to L929 cells in culture were checked by MTT assay. The antitumor activity of GEO was determined by using DLA cell line induced solid tumor and EAC cell line induced ascites tumor model in mice and its comparison with standard anticancer drug cyclophosphamide.

**Results:** GEO showed potent *in vitro* cytotoxic activity against DLA and EAC cell lines. $IC_{50}$ value for DLA cell line was 11 $\mu$g/ml and for EAC cell lines 18 $\mu$g/ml. The $IC_{50}$ of GEO was found to be 41 $\mu$g/ml against the L929 cell lines and to Vero cells was found to be >100 $u$g/ml. The treatment with GEO (500 mg/kg and 1000 mg/kg body weight) significantly reduced the volume of solid tumor development by 54.4% and 62.4% respectively. The life span was increased up to 50% in 1000 mg/kg b. wt GEO treated ascites tumor induced animals.

**Conclusion:** This indicates the significant *in vitro* cytotoxic and antitumor properties of GEO suggesting its potential use as an anticancer agent.

**Keywords**: Ginger essential oil, Cytotoxicity, Antitumorr, MTT assay.

## INTRODUCTION

Cancer is the second leading cause of mortality in the world and projected to become the primary cause of death within the coming years [1]. Chemotherapy is one of the main modalities available for cancer treatment but the current chemotherapeutic drugs available are reported to exhibit toxicity towards normal tissues and possess undesirable side effects. Most cancer chemotherapy regimens make use of highly cytotoxic drugs that target proliferating cell populations. The non-discriminatory use of these drugs leads to severe side effects in normal cells with the high proliferative index, leading to drug resistance and limiting the effective dose of anticancer drug that can be administered. Overcoming these side-effects with anticancer agents from plants may provide a strategy for overcoming the adverse effects of chemotherapy as they are less toxic. Development of naturally derived anticancer drugs, therefore, is crucial, and isolation of novel compounds has become an important part of cancer research.

There is a tremendous historical legacy in folklore use of plant preparations in medicine. Novel, safer and effective compounds with cytotoxic and anti tumour activities will be of immense clinical benefit. A wide variety of biologically active compounds from plants have been used as effective chemotherapeutic agents. Around 60% of the current anticancer drugs, in use today are of natural origin. Vinblastine, vincristine, etoposide, teniposide, taxol, topotecan and irinotecan are some of the approved anticancer drugs.

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 relevant to these diseases. The diverse therapeutic potential of essential oils has drawn the attention of researchers to test them for anticancer activity. Plants of the family *Zingiberacea* are well known for their use as spices and as the remedy for medical ailments in the traditional systems of medicine. The *Zingiberaceae* plants contain essential oils which comprise terpenoids, phenylpropanoids, flavonoids, and sesquiterpenes, that has significant antitumour activity. Bioactive compounds like 6-gingerol, zerumbone, present in this family are known to induce apoptosis in cancer cells. Cytotoxicity and *in vivo* antitumor screening models provide important preliminary data to select compounds with antineoplastic properties for further studies [2]. The present work was carried out to evaluate the antitumor activity of the essential oil extracted from *Zingiber officinale* Roscoe (ginger) both *in vitro* and *in vivo*.

## MATERIALS AND METHODS

### Ginger essential oil (GEO)

GEO was provided by Kancore Ingredients Limited., Angamali, Kerala, India. It was dissolved in hexane (100 mg/10 ml) and 10 $\mu$l of Triton X 100 was added and further evaporated to dryness and finally made up to 10 ml with distilled water. The oil was dissolved in paraffin oil for all *in vivo* studies.

### Chemicals

3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) and Dulbecco's Modified Eagle Medium (DMEM) were obtained from Sigma-Aldrich, St. Louis, USA. Foetal calf serum was obtained from Biological Industries, Israel. All other chemicals and reagents used were of analytical grade.

### Cell lines

The Dalton's Lymphoma Ascites (DLA) and Ehrlich Ascites Carcinoma (EAC) cell lines are maintained at Amala Cancer Research Institute, Thrissur and propagated into transplantable tumors in the peritoneal cavity of female Swiss albino mice. Freshly aspirated cells from the mouse peritoneum were washed with phosphate-buffered saline (PBS) under sterile conditions and their number was determined using a hemocytometer before transplantation. One million cells were transplanted to fresh Swiss albino mice to maintain the cell line. L929 (mouse lung fibroblast) cell line and Vero cell line were obtained from National Centre for Cell Sciences (NCSS), Pune.

### Animals

Male Swiss albino mice (6-8 week old, 25±3 g weight) were purchased from Small Animal Breeding Station, Kerala Agricultural University, Thrissur, Kerala, India. They were housed in well-ventilated polypropylene cages under controlled temperature, and humidity, and were provided with normal mouse chow (Sai Durga Feeds and Foods, Bangalore) and water ad libitum. Animal experiments were conducted after getting prior permission from

Institutional Animal Ethics Committee (IAEC) and as per the instructions prescribed by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India.

**Determination of antitumor activity of GEO**

The antitumor activities of was determined by both *in vitro* as well as *in vivo* methods.

**Determination of *in vitro* cytotoxicity of GEO to DLA and EAC cells**

The tumor cells were washed thrice with phosphate buffer saline. Viable DLA and EAC cells ($1x10^6$ cells in 0.1 ml) were added to tubes containing various concentrations (10, 25, 50, 100 and 200 $\mu$g/ml) of GEO and the volume was made up to 1 ml using phosphate buffer saline (PBS). Tubes were incubated for 3 hrs at 37 °C. After incubation, cell viability/cytotoxicity was determined by trypan blue exclusion method [3]. The percentage of cytotoxicity was given by, (Number of dead cells/Number of dead cells+Number of live cells) ×100.

**Determination of *in vitro* cytotoxicity of GEO to L929 and vero cells in culture**

Cytotoxicity of GEO was also determined using mouse fibrosarcoma L929 cells and normal African green monkey kidney epithelial Vero cells. Cells were seeded in 96 well flat bottom plates (5000 cells/well) and allowed to adhere for 24 h at 37 $^oC$ with 5% $CO_2$ atmosphere. Different concentrations of GEO were added and further incubated for 48 h.

Microculture tetrazolium assay (MTT assay) was carried out according to the method of Campling, *et al.*, 1991 [4]. The plates were centrifuged and the supernatant was removed and then 100 $\mu$l DMSO was added to the cells and the intensity of the blue colour was read at 570 nm using ELISA plate reader.

% cytotoxicity = O. D of control–O. D of treated/O. D of the control × 100

**Effect of GEO on DLA cell induced ascites tumor**

Male Swiss albino mice were divided into five sets of six animals (n = 6) as follows: Group I: DLA cells alone, Group II: DLA cells+Paraffin oil (Vehicle control), Group III: DLA cells+Cyclophosphamide 10 mg/kg body weight, Group IV: DLA cells+GEO 100 mg/kg body weight, Group V: DLA cells+GEO 500 mg/kg body weight and Group VI: DLA cells+GEO 1000 mg/kg body weight. DLA cells were collected from the donor mouse and suspended in PBS. The viable DLA cells were counted (Trypan blue indicator) and adjusted to a concentration of $1 \times 10^7$ cells/ml.

From this 0.1 ml of DLA cells were injected intraperitoneally (i. p.). Oral treatment with GEO was started 24 h after inoculation and continued for 10 consecutive days. The death pattern of animals due to tumor burden was noted and the percentage of increase in lifespan (ILS) was calculated using the formula ((T-C)/T) x 100, where 'T' and 'C' represent the number of days that treated and control animals survived.

**Effect of GEO on DLA cell induced solid tumor model**

Mice were divided into six groups and each group consisting of six animals as the above experiment. DLA cells were aspirated from peritoneal cavity of the tumor bearing mice. All the animals were injected DLA cells ($1 \times 10^6$ cells/mouse) into the right hind limb of the animals intramuscularly. Oral administration of GEO was started 24 h after tumor inoculation. The radii of developing tumors were measured using vernier calipers at 3 days intervals for one month and tumor volume was calculated using the formula,$V= 4/3\ \pi\ r_1^2 r_2$, where '$r_1$' and '$r_2$' represent the major and minor diameter, respectively [5]. This was compared with untreated control (Group I).

**Statistical analysis**

All data were expressed as mean±standard deviation (SD). Significance levels of comparison of differences were determined by one-way ANOVA followed by post-hoc Dunnett's multiple comparison tests using Graphpad Instat 3 software.

**RESULTS**

**Effect of GEO on *in vitro* cytotoxicity**

**Cytotoxicity of GEO towards DLA and EAC cells**

GEO showed significant cytotoxicity towards both DLA and EAC cell lines. The concentration of ginger essential oil required for 50% death of DLA cell lines ($IC_{50}$) was found to 11 $\mu$g/ml and 18 $\mu$g/ml for EAC cell line, respectively (table 1).

**Table 1: Cytotoxicity of ginger essential oil towards DLA and EAC cell lines**

| Concentration ($\mu$g/ml) | Percentage of cytotoxicity | |
|---|---|---|
| | DLA cells | EAC cells |
| 5 | 25 | 14 |
| 10 | 46 | 20 |
| 20 | 100 | 60 |
| 50 | 100 | 84 |
| 100 | 100 | 100 |

One million cells were incubated with various concentrations of ginger essential oil in a total volume of 1 ml for 3 h at 37 °C. After incubation % of cell cytotoxicity was determined by trypan blue exclusion method. All concentrations were done in triplicate.

**Cytotoxicity of GEO towards L929 cells in culture**

The percentage cytotoxicity progressively increased in a concentration dependent manner in MTT assay. The $IC_{50}$ of GEO was found to be 41 $\mu$g/ml against the L929 cancer cell line used (table 2). The $IC_{50}$ of GEO to Vero cells was found to be >100 $u$g/ml (data not shown).

**Table 2: Cytotoxicity of ginger essential oil towards cell L929 cell line**

| Concentration (µg/ml) | Percentage of cytotoxicity |
|---|---|
| 2 | 0 |
| 5 | 9 |
| 10 | 20 |
| 25 | 38 |
| 50 | 57 |
| 100 | 82 |
| 200 | 100 |

Cells were seeded in 96 well flat bottom plates (5000 cells/well) and different concentrations of ginger essential oil added and incubated for 48 h. MTT assay was carried was carried out according to the method of Campling *et al.*, 1991 and absorbance was measured at 570 nm. All concentrations were done in triplicate.

**Antitumor studies**

**Effect of GEO on lifespan of ascites tumor bearing animals**

Lifespan of ascites tumor bearing animals induced by DLA cells was found to be increased by GEO treatment. The animals inoculated with DLA cell lines alone survived for a period of 16±1 days. In 500 mg/kg b. wt group the lifespan was increased to 22±2.5 days (34%) and in 1000 mg/kg b. wt group the lifespan increased to 24±2.7 days (50%). The administration of standard drug cyclophosphamide (10 mg/kg body weight) increased life span to 26±2 days (table 3).

**Effect of GEO on solid tumor development induced by DLA cells**

A significant reduction of solid tumour was found in GEO treated groups when compared with the control. On the 30$^{th}$ day, the tumour volume of the control animals without any drug treatment was found to be 3.75±0.22 $cm^3$, which was significantly higher compared to GEO treated groups 1.97±0.33 $cm^3$(100 mg/kg b. wt), 1.71±0.2 $cm^3$(500 mg/kg b. wt) and 1.41±0.52 $cm^3$ (1000 mg/kg b. wt). In cyclophosphamide (10 mg/kg b. wt) treated group tumour volume was found to be 0.69±0.04 $cm^3$ (fig. 1).

**Table 3: Effect of GEO on the survival rate of ascitic tumor bearing animals**

| Treatment | Mean survival days | % increase in life span |
|---|---|---|
| Control | 16±1 | |
| Vehicle control | 17±1.2 | |
| Cyclophospamide (10 mg/kg b. wt) | 26±2 | 62.5 |
| GEO 100 mg/kg | 19±1 | 18.75 |
| GEO 500 mg/kg | 22±2.5*** | 34.38 |
| GEO 1000 mg/kg | 24±2.7*** | 50 |

Each value represents the mean±SD (n=6). ***p<0.001 compared with vehicle control.

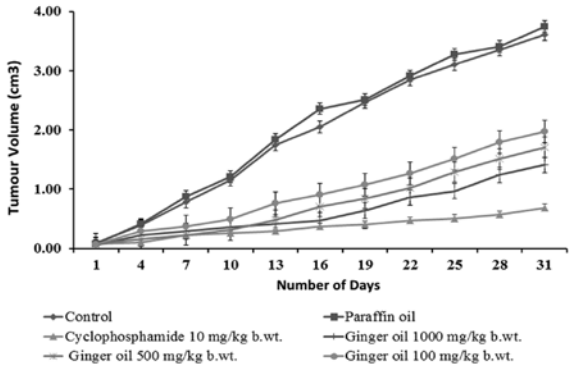


**Fig. 1: Effect of ginger essential oil on solid tumor development**

## DISCUSSION

Combating cancer, which has arisen to become a serious social and economic challenge, is of paramount importance today. A large reservoir of bioactive compounds exists of which only a small percentage have been discovered and used as anticancer agents. A global effort is being made to identify novel anticancer compounds present in plants. Natural products like taxol, vincristine, vinblastine and camptothecin have dramatically improved the effectiveness of the chemotherapy against some of the dreadful cancers [6]. In the present study, the cytotoxic potency of GEO was confirmed by *in vitro* cytotoxic assay methods. Concentration needed for 50% cytotoxicity to DLA cells was 11 $\mu$g/ml and 18 $\mu$g/ml to EAC cells. GEO exhibited potent cytotoxicity (41 $\mu$g/ml) during MTT assay in L929 cancer cell line. However, it was found to be non cytotoxic to Vero cell lines the $IC_{50}$ being >100 $u$g/ml. The low cytotoxic effect on non-cancerous cell line Vero indicates that GEO exhibits cytotoxicity specifically to cancer cells only but not in normal cells.

Lymphoma is a disease of the lymphocytes in the lymphatic system, which includes the spleen, thymus, as well as other lymphatic tissues. Both EAC and DLA are rapidly growing transplantable tumor cells with aggressive behavior [7]. Dalton's ascites lymphoma is transplantable, poorly differentiated malignant tumor which appeared originally as Lymphoma in a mouse. It grows as both solid and ascitic forms [8]. The tumor implantation initiates a local inflammatory reaction, with increasing vascular permeability, which results in an intense ascetic fluid accumulation [9]. Ascitic fluid constitutes the direct nutritional source for tumor cells [10]. In untreated DLA tumor bearing mice, an increase in ascitic tumor volume was observed. GEO treatment (1000 mg/kg b. wt) showed a 50% increase in the life span of the animals induced with DLA. These results indicate that GEO reduced the ascitic fluid formation and arresting the tumor growth and thus increasing the life span of DLA bearing mice. As per the NCI criteria, an ILS exceeding 25% indicates that the drug has significant antitumor activity.

GEO treatment also decreased the volume of the solid tumor in mice in a dose dependent manner. GEO decreased the tumor volume by 54.4% and 62.4% respectively when administered at concentrations of 500 and 1000 mg/kg body weights to animals after implantation of DLA cell line intramuscularly.

The reliable criteria for judging the value of any anticancer drug is the prolongation of life span, and reduction of solid tumor volume [11, 12]. Treatment with GEO reduced the tumor volume, viable tumor cell count, and increased the life span of tumor bearing mice. From the results obtained, we can conclude that GEO possess profound antitumor activity as observed by *in vitro* cell culture experiments as well as *in vivo* antitumor studies. Previous studies have indicated that sub acute oral administration of GEO at 1000 mg/kg b. wt (unpublished) and chronic administration at 500 mg/kg b. wt has proven to be non-toxic to rats [13]. Ginger essential oil has also proved to be cytotoxic to MCF-7, PC-3 and A-549 cell lines [14]. Antitumor and cytotoxic properties of GEO may be due to its bioactive constituents. More studies are needed in order to elucidate the mechanism of action of GEO.

## CONCLUSION

In this study, GEO exhibited significant cytotoxic activity in DLA and EAC cell lines *in vitro*. MTT assay using L929 and Vero cells indicates that GEO exhibits significant antiproliferative activity selectively to cancer cells only. Treatment with GEO significantly reduced the volume of solid tumor development and increased the lifespan of ascites tumor induced animals significantly. These results indicated the potent cytotoxic and antitumor activity of GEO and its potential to be developed as an anticancer agent. Further work is required to understand the molecular mechanism of action of GEO.

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## CONFLICT OF INTERESTS

No conflict to disclose

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