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Ginger Extract Antimutagens as Cancer Chemopreventive Agent Against Ehrlich Ascites Carcinoma

Zeinab E. Hanafy

Department of Zoology, Faculty of Science (Girls), Al-Azhar University, Cairo, Egypt

Abstract: Ginger (Zingiber officinale Roscoe, Zingiberaceae) is a commonly used medicinal herb throughout the world. Ginger (*Zingiber officinale* Rosc) is a natural dietary component with antioxidant and anticarcinogenic properties. Although some studies have demonstrated its antitumour activities on cancer cells both *in vitro* and *in vivo*. In the present study, the influence of Ginger extract on the mice inoculated with Ehrlich ascites was investigated. Cytogenetical studies, micronucleus test in bone marrow cells and DAN fragmentation assay in liver cells was determined in the presence or absence of Ginger. The study was performed on four groups of female mice, i.e. control group, Ginger group(animal received a daily oral dose 100 mg /Kg body wt.).In third group, mice were intraperitoneally (*i.p.*) inoculated with 2.5x 10^6 Ehrlich ascites cells. In the fourth group, animal received oral daily ginger on day 2 after inoculation the animals Ehrlich ascites cells. Result reveled that the micronuclei cells increase in mice inoculated with Ehrlich ascites. The ginger extract reduced the incidence of micronucleated cells formation induced by Ehrlich ascites cells inoculation as almost normal or less than control values. Also, there was an increased incidence of DNA fragmentation in mice inoculated with Ehrlich ascites. When ginger was used after inoculated with Ehrlich ascites cells the percentage of DNA fragmentation was highly decrease. It was concluded that the ginger extract may have a chemotherapeutic effect in the treatment of cancer. The use of dietary agents such as ginger may have potential in the treatment and prevention of cancer.

Key words: *Zingiber officinale* · DAN fragmentation · Micronucleus test · Female mice

INTRODUCTION

Environmental carcinogens and mutagens induce DNA and chromosomal damage. Assessment of genotoxicity can be performed at different steps of the interaction as well as the effects of the mutagen on DNA.

Large number of studies has revealed that a regular consumption of fruits and vegetables provide good protection against cancer at many sites and some of the foods and herbs contain a host of cancer-protective phytochemicals [1]. These beneficial compounds alter metabolic pathways and hormonal actions that are associated with the development of cancer, stimulate the immune system and have antioxidant activity [2].

Mutagenicity, clastogenicity, cytotoxicity and carcinogenicity are inhabited by antioxidant compounds found in abundance in plants [3]. Most chemopreventive compounds and their analogs and derivatives are initially of plant origin and inhibit spontaneous and chemical mutagenesis in a variety of *in vitro* and *in vivo* test systems [4]. The screening of crude plant extracts for antimutagenic and anticarcinogenic activity has led to the identification of a variety of compounds[5, 6].

In recent times, spices are being discovered to have antimutagenic and anticarcinogenic potentials and there has been substantial evidence of data, supporting that dietary factors have a profound effect on prevention and etiology of human cancer [7].

Plant of ginger (*Zingeiber officinale* Roscoe, *Zingiberaceae*) family is one of the most highly consumed dietary substances in the world. Ginger (*Zingiber officinale)* is widely used all over the world as a spice and condiment in daily cooking. It is a natural food component with many active phenolic compounds such as shagaol and gingerol and it has been shown to have antioxidant effects [8,9], anti-angiogenesis [10], anti-inflammatory [11,12], anti-artherosclerotic properties [13], and anti-cancer[9,14]. In an *in vitro* experiment on ginger it was shown that ginger has potential to inhibit carcinogen induced cell damage by benzo(a) pyrene as measured by comet assay [15].

Corresponding Author: Zeinab E. Hanafy, Department of Zoology,
Faculty of Science (Girls), Al-Azhar University, Cairo, Egypt

In the present study, the potential anti-cancer effects of ginger extract was tested by using micronucleus test and DNA fragmentation.

MATERIALS AND METHODS

Preparation of Plant Extract: The air-dried and powdered plant materials (10 g) were extracted with 400 ml methanol (CH_3OH) by Soxhlet extraction for 8 hours. The residue was dried over night and then extracted with 250 ml water (H_2O) by using a shaking water-bath at 70°C for 2 hours. The obtained methanolic and water extracts were filtered and evaporated by using a rotary evaporator and freeze dryer. The dried extracts were stored at-20°C until used [16].

Tumor Cell Line: A line of Ehrlich Ascites Carcinoma (EAC) obtained from Egyptian National Cancer institute, Cairo University. The parent line was supplied through the courtesy of Dr. Gklein, Amsterdam, Holland. The tumor line was maintained in female Swiss albino mice by weekly interperitoneal injection of 2.5x 10^6 cells/mouse according to the method recommended by the Egyptian National Cancer institute, Cairo university.

Such developed tumor is characterized by its moderate rapid growth which could not kill the animal due to the accumulation of Ascites before injection using the bright line haemocytometer and dilutions were made by physiological saline and desired number of cells were injected in a volume of 0.5 ml.

Animals: Eighty female mice (18-20 g.) were obtained from the house of the experimental animals, the National Research Center, Cairo, Egypt. The animals were kept under normal healthy laboratory conditions inbred colony was used in the present study. Animals were provided with standard rat feed (procured from Animal Nutrient Co., Cairo) and water *ad libitum* and were maintained under controlled conditions of temperature and light (Light: dark, 10 hrs: 14 hrs.). 5 animals were housed in a polypropylene cage with locally procured paddy husk (Oryza sativa) as bedding throughout the experiment. Tetracycline-containing water (0.13 mg/ml) was provided once a fortnight and was given as a preventive measure against infections. Animal care and handling were performed according to the guidelines set by the World Health Organization (WHO), Geneva, Switzerland and the ETC (Ethical Committee National Research Center), Cairo, Egypt.

Experimental Design: Mice were divided into four groups to carry out the experimental study. These groups were as follows:

- First group: received diet and water *ad libitum* and served as control
- Second group: animals in this group received oral daily dose of ginger only (100 mg /Kg body wt.).
- Third group: mice were intraperitoneally (*i.p.*) inoculated with 2.5x 10^6 Ehrlich ascites cells.
- Fourth group: animal received oral daily ginger (100 mg /Kg body wt.) on day two after inoculation with Ehrlich ascites cells.

Each group was divided into two equal subgroups.

The first subgroup was sacrificed after 7 days, while the second subgroup, was sacrificed after 14 days.

The bone marrow was collected for micronucleus test and the tissue samples from livers were collected for DNA fragmentation.

The Micronucleus Test: The bone marrow of five animals of control and all the treated groups were extracted smear preparations made by using fetal calf serum according to the method of Salamone *et al.* [17].

DNA Fragmentation Assay: The method of DNA fragmentation was carried out according to Burton [18] and Perandones *et al.* [19].

Statistical Analysis: The obtained data were subjected to analysis of PRIMER Ver 5.0 according to Bary-curtis Similarity Inedex [20].

RESULTS

Micronucleus Test: In the present study, animals only received oral daily ginger (100 mg /Kg body wt.) slightly low incidence of the micronucleated cells in bone marrow cells was found than control, the micronucleated cells yield was 2.6 after 7days and 2 after 14days (Tables, 1,2 and Fig,1).

With Ehrlich ascites cells inoculated in mice, the micronucleated cells yield sharply increased to 19.6 after 7days and 24 after 14days (Table, 1, 2 and Fig,1). The ginger extract reduced the micronucleated cells induced by inoculated Ehrlich ascites cells whereas it was almost normal or less than control values.

Table 1: The effect of Ehrlich ascites cells with or without Ginger (Zingiber officinale) on micronuclei (MN) formation in bone marrow cells in female mice

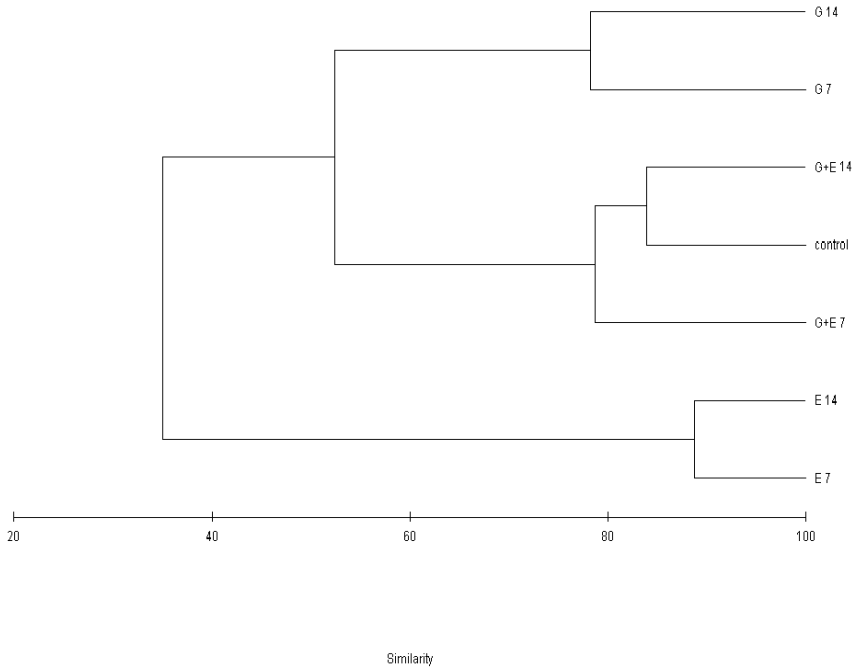
Groups	MN frequency	Mean
control	7, 8, 4, 7, 6	6.4
Ginger (7 days)	1, 3, 3, 3, 3	2.6
Ginger (14 days)	2, 2, 3, 2, 1	2
Ehrlich (7 days)	24, 15, 21, 20, 18	19.6
Ehrlich (14 days)	27, 26, 22, 28, 20	24
Ginger+ Ehrlich (7 days)	4, 7, 7, 7, 10	7
Ginger+ Ehrlich (14 days)	9, 4, 5, 8, 4	6

Table 2: Similarity between different groups on micronuclei (MN) in bone marrow cells in female mice

Groups	control	Ginger(7 days)	Ginger(14days)	Ehrlich(7 days)	Ehrlich(14days)	Ginger+ Ehrlich(7 days)	Ginger+ Ehrlich(14days)
Control							
Ginger(7 days)	57.77778						
Ginger(14days)	47.61905	78.26087					
Ehrlich(7days)	49.23077	23.42342	18.51852				
Ehrlich(14days)	41.29032	19.11765	15.03759	88.68778			
Ginger+Ehrlich(7 days)	83.58209	54.16667	44.44444	52.63158	44.3038		
Ginger+Ehrlich(14days)	83.87097	60.46512	50	46.875	39.21569	73.84615	

Table 3: The effect of Ehrlich ascites cells with or without Ginger (Zingiber officinale) on DNA fragmentation in female mice

Groups	DNA fragmentation %	Change %
Control	5.6 %	---
Ginger (7 days)	9.7%	+ 4.1
Ginger (14 days)	10.8 %	+ 5.2
Ehrlich (7 days)	21.7 %	+ 16.1
Ehrlich (14 days)	26.9 %	+ 21.3
Ginger+ Ehrlich (7 days)	14.3%	+ 8.7
Ginger+ Ehrlich (14 days)	12.3 %	+ 6.7



G7: Ginger (7 days) G14: Ginger (14 days) E7: Ehrlich (7 days) E14: Ehrlich (14 days)
G+E7: Ginger+ Ehrlich (7 days) G+E14 Ginger+ Ehrlich (14 days)

Fig. 1: Dendrogram represent similarity between different groups of female mice

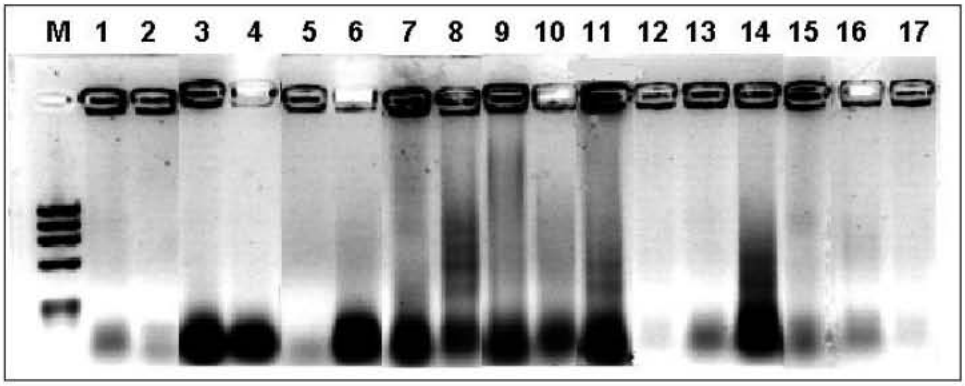


Fig. 2: The effect of Ehrlich ascites cells with or without Ginger (Zingiber officinale) on DNA fragmentation in female mice.
Lane M: DNA molecular weight marker
Lane 1 : Control (7 days) Lane 7, 8 : Ehrlich (14days)
Lane 2 : Control (14 days) Lane 9, 10, 11: Ehrlich (7 days)
Lane 3, 4: Ginger (7 days) Lane12, 13, 14: Ginger+ Ehrlich (7 days)
Lane 5, 6: Ginger (14 days) Lane15, 16, 17: Ginger+ Ehrlich (14 days)

DNA Fragmentation: In mice intraperitoneally inoculated with Ehrlich ascites cells, marked DNA fragmentation after 7days (21.7%) and 14 days (26.9) were recorded as compared to control untreated mice (5.6%). In simultaneous treatment mice with Ginger and Ehrlich ascites cells, the incidence of induced DNA fragmentation decreased after 7days (14.3%) and 14 days (12.3 %). However, when animal treated with Ginger only DNA fragmentation slightly increased than control. (Table, 3 and Fig,2).

DISCUSSION

Among the most commonly included agents in foods, are flavonoids and phenolic substances. Hence, phenolic compounds take important parts of the human diet. In addition, current interest is raised up by many observations that dietary phenolic compounds have various activities such as antioxidant, antiinflammation and anti-carcinogenesis. Phenolic compounds comprise one of the largest and most ubiquitous groups of plant metabolites. They are formed to protect the plant from photosynthetic stress, reactive oxygen species (ROS), wounds and herbivores [21].

Accumulating evidence suggests that many dietary factors may be used alone or in combination with traditional chemotherapeutic agents to prevent or treat cancer. The main advantage of using natural or dietary compounds as an anti-cancer remedy is that they seem to have low toxicity and show very few adverse side effects.

From a large number of Zingiberaceae species that have been used for culinary and /or medicinal purposes, only few members have studied for their potential anticancer activity. Murakami *et al.* [22] reported that Zerumbone, which was isolated from *Zingier aromaticum* and *Zingier zerumber,* had an antiproliferative effect on various human colonic adinocarcinoma cell lines. However, the molecular mechanisms by which they exert their antitumorogenic effects are unknown [23].

In the current study, animals received oral daily ginger, there was showed slightly low incidence of micronucleated cells in bone marrow cells than control. however DNA fragmentation slightly increased. Nirmala *et al.* [24] indicated that Ginger extract tested at various concentrations did not show any adverse effects. Ginger extract did not exert cytogenetic effect on human PBL at concentrations up to 0.4mg as evaluated by the CBMN assay. Ali *et al.* [25] reported that the main pharmacological actions of ginger and isolated compounds include immuno-modulatory, anti-tumorigenic, anti-inflammatory, anti-apoptotic, anti-hyperglycemic, anti-lipidemic and anti-emetic actions. Ginger is a strong anti-oxidant substance and may either mitigate or prevent generation of free radicals. It is considered a safe herbal medicine with only few and insignificant adverse/side effects.

The present data revealed that inoculation of mice with Ehrlich ascites cells sharply increased micronucleated cells yield after 7days and 14days. However, following treatment with ginger extract the micronucleated cells induced reduced to normal or less than control values.

Nirmala *et al.* [24] observed increased (P<0.001) incidence of micronucleus following incubation of blood cells with Trans stillbene oxide (TSO). On the contrary, in the presence of ginger and TSO exposure, reduction in the level of spontaneously occurring micronuclei was observed. A decrease was noted with the increasing levels of ginger extracts present in incubation medium. Therefore, ginger might have therapeutic value as a possible chemopreventer by virtue of its anticytotoxic property. Mayer *et al.* [26] have demonstrated positive correlation between lipid peroxidation and increased MN formation in lymphocytes. Oxidative stress due to free radical production and subsequent breakdown of antioxidant defense is one of the positive factors to induce chromosomal breakage and MN formation. The enhanced activity of GST and QR in target tissues like livers, lungs and kidney in ginger treated rats suggest that antigenotoxic effect could be through detoxification pathway [24].

In the present study, results showed that Ehrlich ascites cells caused marked DNA fragmentation after 7days and 14 days, however mice treated with Ginger highly decreased Ehrlich ascites cells induced DNA fragmentation after 7days and 14 days. Lu *et al.* [27] demonstrated that Ginger oil might decrease light value compared with the control group and inhibited erythrocyte oxidation damage. Compared with that in control group, DNA damage reduced significantly in the protected groups. Ginger oil has dominantive protective effect on DNA damage induced by H2O2. Ginger oil might act as a scavenger of oxygen radical and might be used as an antioxidant.

Antimutagens and anticarcinogens are natural or synthetic substances able to inhibit or to reduced spontaneous or induced DNA alteration. They react directly with mutagens or on the process of their activation [28]. Dietary antimutagens may provide means of slowing progression towards cancer and be more acceptable to the population. Antioxidants have been suggested to scavenge free radicals and prevent their interaction with cellular DNA [29]. Some pungent constituents of ginger have potent antioxidant and anti-inflammatory effects and some of them exhibit antitumour promotional activity in experimental carcinogenesis [30,31].

Akhurst *et al.* [32] showed that ginger extract was able to reduce the incidence of liver neoplasms in rats. The anti-cancer effect exhibited by ginger on liver cancer cells is mediated by inflammatory markers NFêB and TNF-α. Oval cell proliferation precedes neoplasia in many rodent models of hepatocellular carcinoma and prevention of this proliferative response can reduce the risk of subsequent carcinoma. *In vivo* studies in rats fed with ginger also showed that it has strong antimutagenic and antioxidant potential [33,34]. Rhode *et al.* [35] Ginger inhibits growth and modulates secretion of angiogenic factors in ovarian cancer cells. The use of dietary agents such as ginger may have potential in the treatment and prevention of ovarian cancer.

In conclusion, Ginger inhibits growth in Ehrlich ascites cells. The use of dietary agents such as ginger may have potential in the treatment and prevention of cancer.

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