Cytogenetics Changes on Cancer Cells as Affected by Ginger Extracts

Zeinab E. Hanafy

Department of Zoology, Faculty of Science (Girls), Al-Azhar University, Cairo, Egypt
zainabhanafy@gmail.com

Abstract: A wide variety of phenolic substances derived from spice possess potent antimutagenic and anticarcinogenic activities**.** Some of these phenolic substances are present in ginger, possessing strong anti-inflammatory and anti-oxidative properties as well as exert substantial anti-carcinogenic and anti-mutagenic activities. The present study was conducted to examine the in vivo cytogenetic effect of ginger extract on Ehrlich ascites cell inoculated in female mice.

This study was performed on two groups of female mice. The first, one was inoculated intraperitoneally (*i.p.*) with 2.5x 10^6 Ehrlich ascites cells. However, the second one received oral daily ginger (100 mg /Kg body wt.) on day two of inoculating animals with Ehrlich ascites cells.
Results revealed that various types of chromosomal aberrations in Ehrlich ascites cells were detected. These aberrations were manifested in either numerical or structural aberrations. Ehrlich ascites cells contain different number of chromosomes ranging form 26 to 125 with an increase in micronuclei cells and incidence of mitotic index**.** The ginger extract increased the percentage of diploid cells (normal morphology), In addition, a reduction in micronuclei cells and mitotic index in Ehrlich ascites cells were detected. Also, a reduction in chromosomal aberration of Ehrlich ascites cells was achieved. The reduction of abnormalities in tumor cells by the extracts may stimulate the cells to divide normally or go to die (through apoptosis) if they cannot remove chromosomal abnormalities.

It was concluded that ginger extract may have a chemotherapeutic effect on Ehrlich ascites cells. The extract greatly changed tumor cells to diploid normal cells. The use of dietary agents such as ginger may have potency for the treatment and prevention of cancer. [Journal of American Science 2010; 6(8):525-539]. (ISSN: 1545-1003).

Key words: *Zingiber officinale*, Ehrlich ascites cell, chromosomal aberration, micronuclei cells.

1. Introduction:

Epidemiologic evidence suggested that regular consumption of fruits, vegetables, and whole grains may reduce cancer risk in some individuals. This association has been attributed to these foods being rich sources of numerous bioactive compounds (Milner,2004). Bioactive components present in fruits and vegetables can prevent carcinogenesis by blocking metabolic activation, by increasing detoxification, or by providing alternative targets for electrophilic metabolites (kuo *et al.,* 2005). Numerous constituents of plant foods, including flavonoids (such as quercetin, rutin, and genistein), phenols (such as curcumin, epigallocatin-3-gallate and resveratrol), isothiocyanates, allyl sulfur compounds, indoles, and selenium have been found to be potent modulators of detoxification enzymes *in vitro* and in preclinical models (Milner ,2001 and Keum *et al.,* 2004).

The effect of plant extracts as antitumors was widely studied due to their low toxicity and side effect. The inhibition of ascites tumor cells by Nigella sativa seed extracts was investigated (Musa *et al.,*2004). Willow extracts antitumor activity which is due to the presence of Salicin (Zahran *et al.,*2005). Curcuma longa extracts and active constituents have a potential role in the prevention of cancer and the management of infectious and chronic diseases (Ahmad *et al.,* 2008).

The metaphase of tumor cells was highly arrested by vincristine and vinblastine as alkaloids which have antimitotic effect (EL-Mrezabani *et al.,* 1979b).The abnormalities in chromosomes of tumor cells such as sister chromatid change and aberrations (Duthie *et al.,* 1997; Gonalez *et al.,* 1997) and DNA fragmentation (Royman and Ruddon, 1995) by natural extracts were illustrated. The induction of apoptosis in the cancer cells by natural plant extracts was studied by (Pirianov *et al*., 1998 ;Filion *et al*., 1998 and Choudhury *et al.,* 2010).

Mutagenicity, clastogenicity, cytotoxicity and carcinogenicity are inhibited by antioxidant compounds found in abundance in plants (Jeyakumar *et al.,* 1999 and Surh, 2003). Most chemopreventive compounds and their analogs or derivatives are initially of plant origin and inhibit spontaneous and

chemical mutagenesis in a variety of in vitro and in vivo test systems (Xifeng *et al.,* 2007).

Many herbs and spices are known to possess an array of biochemical and pharmacological activities including antioxidant and anti-inflammatory properties that were believed to contribute to their anticarcinogenic and antimutagenic activities (Hung *et al.,* 2006). Tumor promotion is closely linked to inflammation and oxidative stress; so compounds that exhibit anti inflammatory and/or antioxidant properties could acts as anticarcinogenic agent (Masuda *et al.,* 2004).Some phenolic substances present in ginger(*Zingiber officinale Roscoe, Zingiberaceae*), generally, possess strong anti-inflammatory, anti-oxidative and anti-mitotic properties and exert substantial anti-carcinogenic and anti-mutagenic activities (Surh, 2002 ; Bode, 2003; Kim *et al.,*2005a ; Vijayapadma *et al.,* 2007 and Choudhury *et al.,* 2010).

This study amid to evaluate the effect of ginger extract on characteristics of ascitic Ehrlich tumor cells by using chromosome aberration assay, mitotic index and micronucleus test.

2. Materials and Methods:

Preparation of plant extract

It is conducted according to Mothana *et al*. (2009) where air-dried and powdered plant materials (10 g) were extracted with 400 ml methanol (CH_3OH) by Soxhlet extraction for 8 hours. Residue was dried overnight 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.

Tumor Cell Line

A line of Ehrlich ascites carcinoma (EAC) obtained from Egyptian National Cancer institute, Cairo University. 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 numbers of cells were injected in a volume of 0.5 ml.

Animals:

Forty female mice (18-20 g.) were obtained from experimental animal house found in National Research Center, Cairo, Egypt. 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) with water *ad libitum* and were maintained under controlled conditions of temperature and light (Light: dark, 10 hrs: 14 hrs.). Five 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 two groups:

1-Ehrlich group: mice were intraperitoneally (*i.p.*) inoculated with 2.5x 10^6 Ehrlich ascites cells.

2-Ehrlich and ginger group: animal received oral daily ginger (100 mg /Kg body wt.) on day two after inoculation with Ehrlich ascites cells.

Collection and preparation of Ehrlich ascites cells

After 7 and 14 days of tumor implantation, animals were necropsied. Ascitic liquid was collected in order to chromosome preparation and micronucleated cells. Smears were carried out from the cell suspension obtained from each animal and then they were submitted to Giemsa stain in order to determine mitotic index.

Chromosome preparation: was conducted according to Evans (1987):

Animals were sacrificed and Ehrlich ascites cells were collected. Ehrlich ascites cells was subjected to colchicine treatment (0.05 solution, 0.1 ml /culture), hypotonic treatment (KCl, 5.6 g/l), fixed in acetomethanol, spread and stained with Giemsa stain

Mitotic Index:

1000 cells per animal were counted and the number of dividing cells, including prophases and metaphases, was determined.

The micronucleus test: was conducted according to the method of Salamone *et al*., 1980.

Ascitic liquid was collected from five animals of both groups and smear preparations were made by using fetal calf serum.

Statistical analysis:

Obtained data were subjected to analysis of PRIMER Ver 5.0 according to Bary-curtis Similarity Inedex. and ANOVA-test according to Snedecor & Cochran (1980) at probability 0.01

3. Results:

Chromosome number aberrations

As shows in Table (1); on day 7& 14; in Ehrlich group, Ehrlich ascites cells contain different number of chromosomes ranging form 26 to 125 chromosomes (Plate 1, 2 & 3). The stem cells of this tumor had 46 rod – shaped chromosome (Plate3- a). In Ehrlich and ginger group, ginger extract significant decrease the variations of chromosome number in Ehrlich ascites tumor cells. The ginger extract a significant increase diploid Ehrlich ascites cells (46 chromosomes).

Structural chromosomal aberrations

Data cited in table (3) show the percentage of the Structural chromosome aberration in diploid Ehrlich ascites cells (46 chromosomes) on day 14. These aberrations included breaks, deletions, rings, end to end association, centric fusion and fragment (Plate 4&5). Centric fusion type observed in Ehrlich group with percentage more than all types of chromosome aberration (25%). Addition of ginger extract reduced chromosome aberration in Ehrlich ascites cells. Centric fusion type highly decreases in Ehrlich ascites cells treated with ginger extract (8%). Also the percentage of other type of chromosome aberration was decreased except in break type were increased (Fig. 3).

Mitotic Index

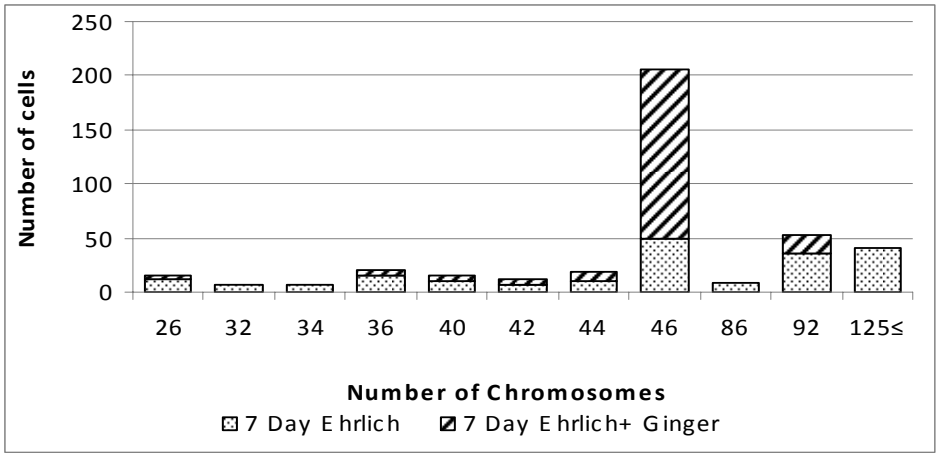
Cytological studies using Giemsa staining methods revealed a significant increase in number of mitotic cells in Ehrlich ascites cells at 7 and 14 days. While ginger extract induces a significant decrease in mitotic cells in Ehrlich ascites tumor cells (Table 4& Fig. 4).

The micronucleus test:

On analyzing the frequency of micronucleated cells in Ehrlich ascites tumor cells (Table 4), it was found that significant increase in the frequency of micronucleated cells in Ehrlich group. When Ehrlich ascites cells treated with ginger extrac significant decrease in the micronucleated cells was observed (Fig. 5).

Table (1): Distribution of chromosome numbers in Ehrlich ascites cells treated with orwithout ginger extract after 7 and 14days

Groups		number of chromosomes										
		26	32	34	36	40	42	44	46	86	92	125≤
Ehrlich 7days	total	12	6	6	16	10	6	10	50	8	36	40
	%	6	3	3	8	5	3	5	25	4	18	20
Ehrlich+	total	4	0	0	4	6	6	8	156	0	16	0
Ginger 7days	%	2	0	0	2	3	3	4	78	0	8	0
Ehrlich 14days	total	6	3	5	7	30	0	19	117	0	13	0
	%	3	1.5	2.5	3.5	15	0	9.5	58.5	0	6.5	0
Ehrlich+	total	5	0	0	6	8	0	8	164	0	9	0
Ginger 14days	%	2.5	0	0	3	4	0	4	82	0	4.5	0



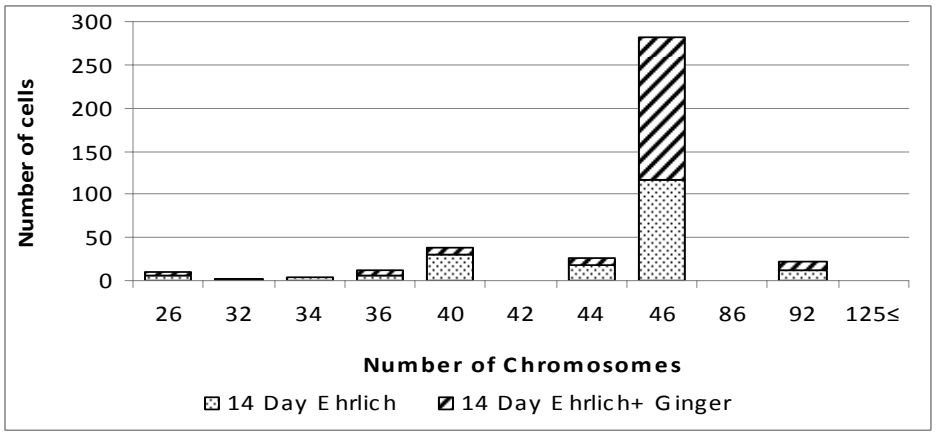


Fig. 1: Distribution of chromosome number in Ehrlich ascites cells treated with ginger extract after 7 and 14days.

Table (2): Distribution of chromosome number in Ehrlich ascites cells (normal and abnormal) treated with or without ginger extract after 14days

Groups		number of chromosomes							
		26	32	34	36	40	44	46	92
Ehrlich 14days	normal	5	2	3	3	17	13	54	4
	abnormal	1	1	2	4	13	6	63	9
	total	6	3	5	7	30	19	117	13
	%	3	1.5	2.5	3.5	15	9.5	58.5	6.5
Ehrlich+ Ginger 14days	normal	4	0	0	3	6	5	120	4
	abnormal	1	0	0	3	2	3	44	5
	total	5	0	0	6	8	8	164	9
	%	2.5	0	0	3	4	4	82	4.5

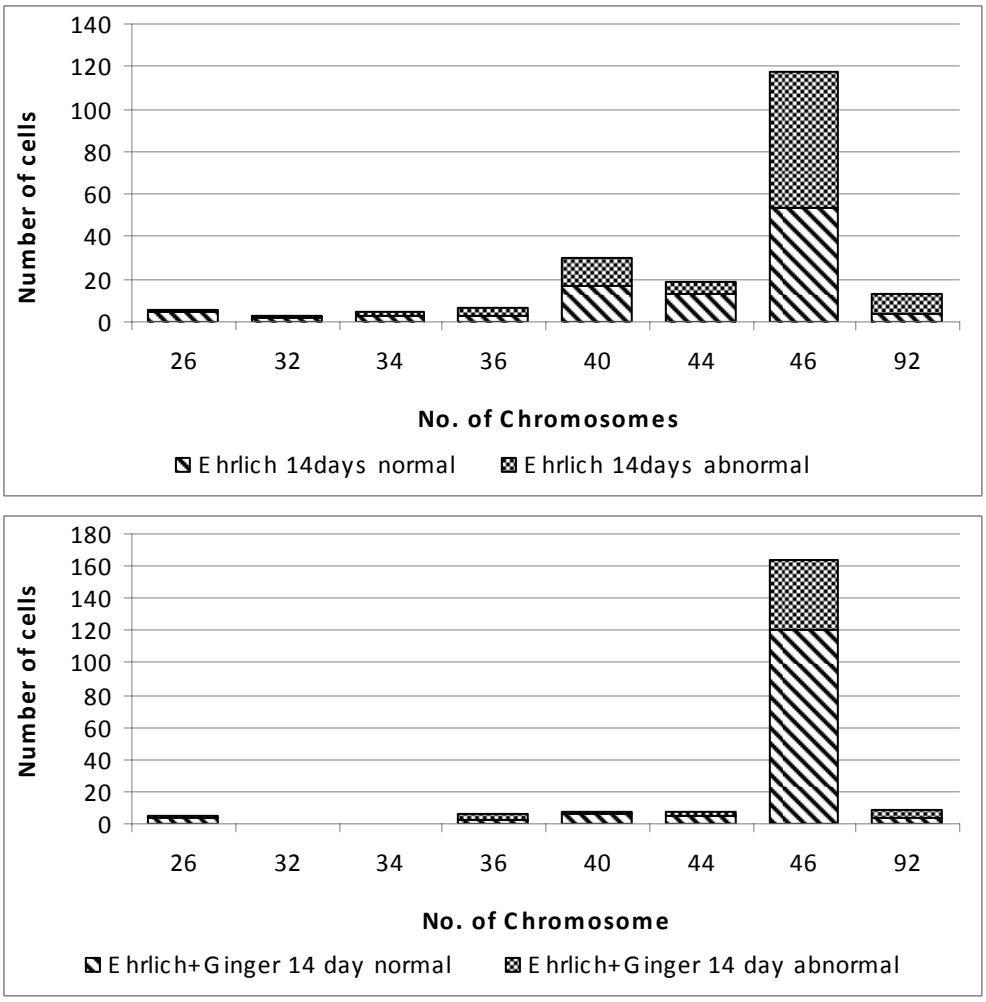


Fig. 2: Distribution of chromosome number in Ehrlich ascites cells (normal and abnormal) treated with or without ginger extract after 14days

Table 3: Structural chromosomal aberration in diploid Ehrlich ascites cells (46 chromosomes) treated with or without Ginger extract.

Groups	Abnormal cells							Normal cell
	Structural chromosomal aberrations							
	Break	Deletion	Ring	End to End association	Centric fusion	fragment	More aberrations in one cell	
Ehrlich (14 days)	18	8	4	2	50	14	12	92
	9%	4%	2%	1%	25%	7%	6%	46%
Ehrlich+ Ginger(14 days)	22	2	2	2	16	2	8	146
	11%	1%	1%	1%	8%	1%	4%	73%

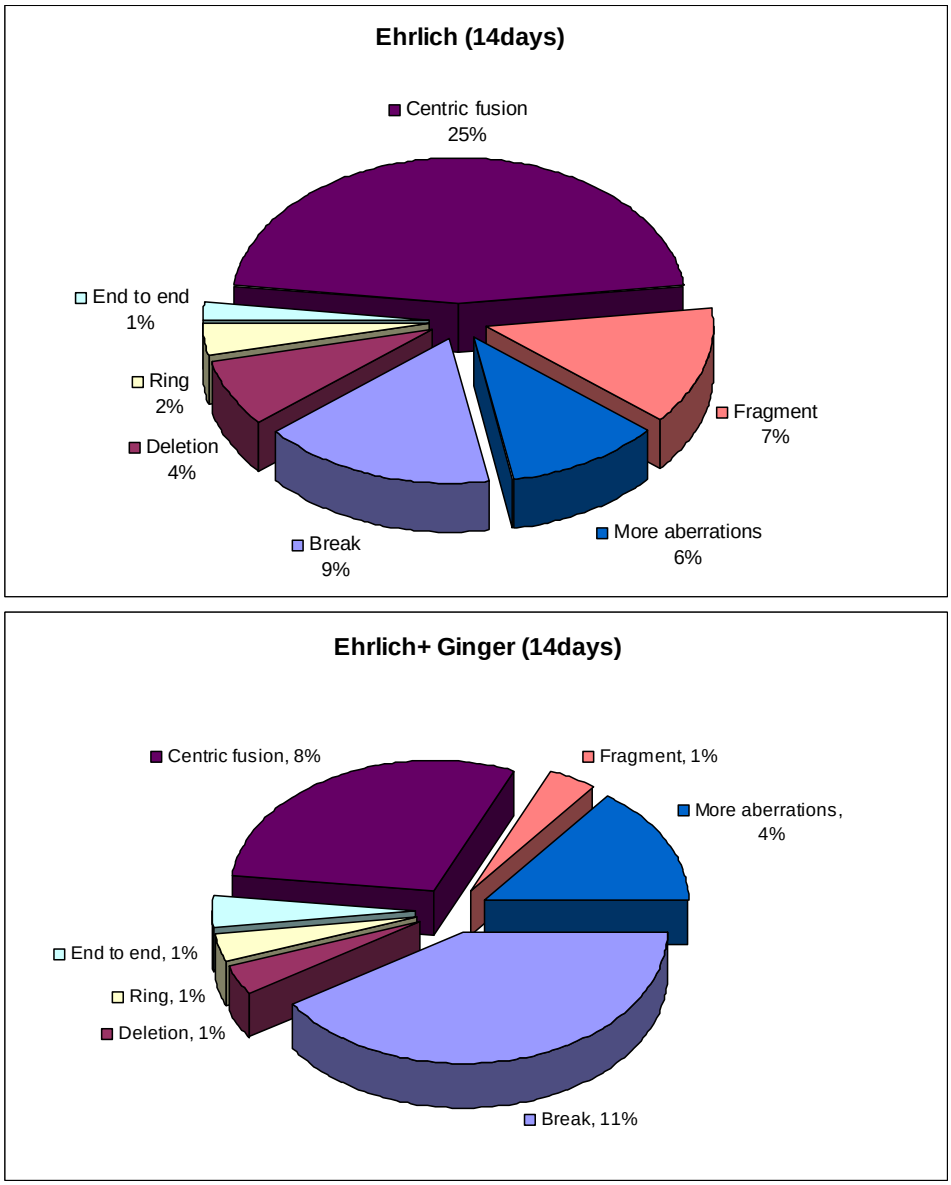


Fig. 3: Structural Chromosomal aberration in diploid Ehrlich ascites cells (46chromosomes) treated with or without Ginger extract after 14days

Table 4: Mean of mitotic index and micronuclei cell in Ehrlich ascitic tumor cells treated with or without Ginger extract after 7 and 14days.

Groups	Mean	
	Mitotic index	Micronuclei cell
Ehrlich (7days)	216	78
Ehrlich +Ginger (7days)	33	19
Ehrlich (14days)	238	91
Ehrlich +Ginger (14days)	74	23

Each point on the chart is 1000 cell counted from each animal.

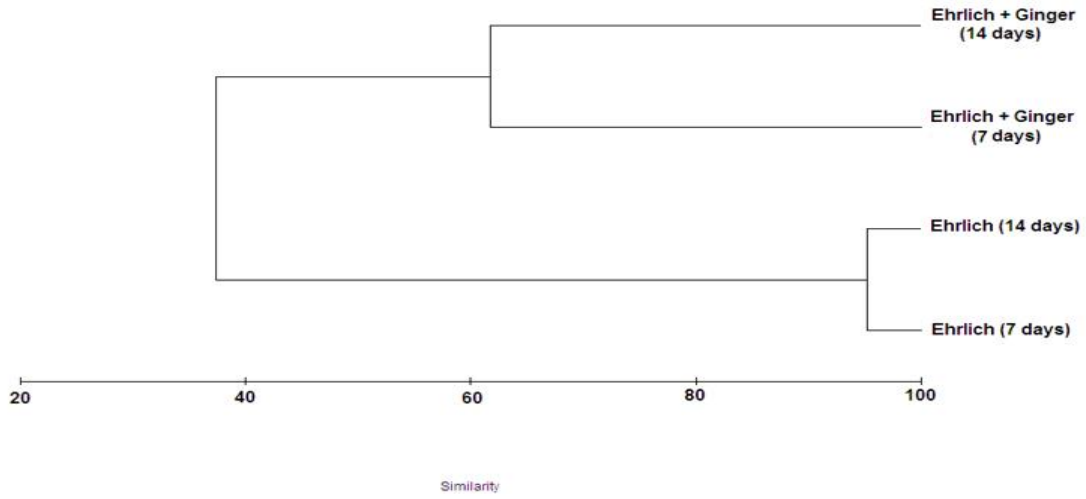


Figure 4: Dendrogram represent similarity of mitotic index between different groups after 7 and 14 days

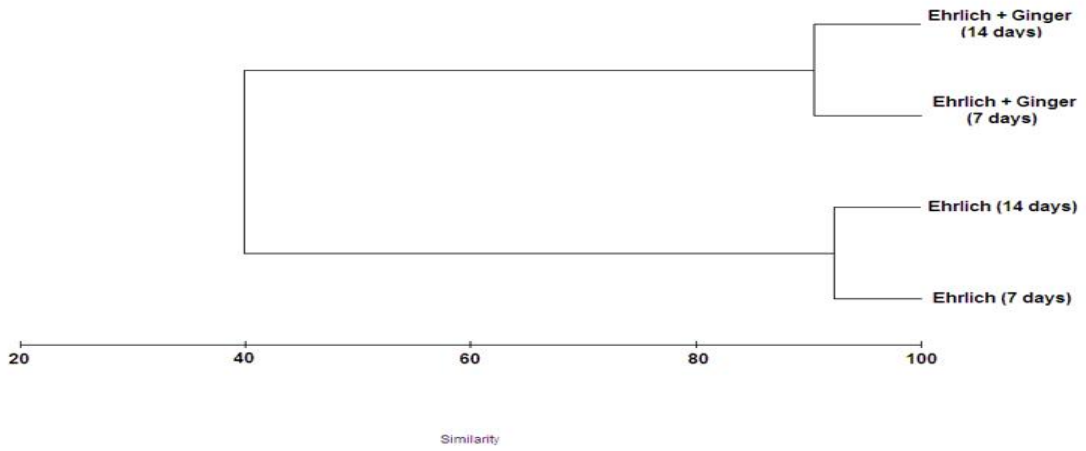
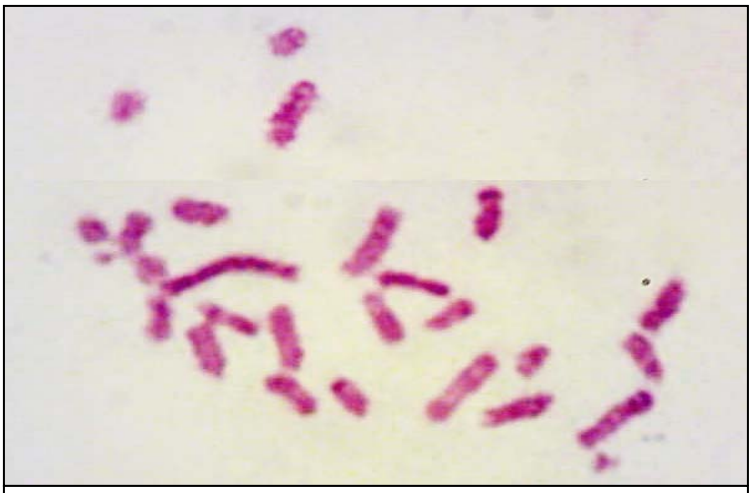
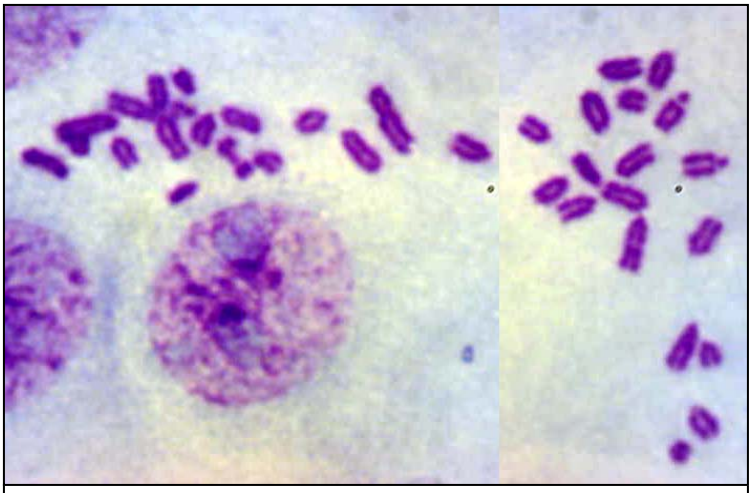


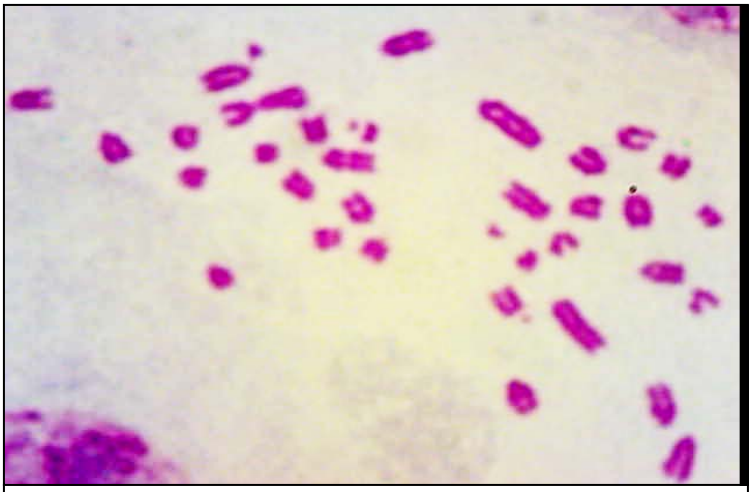
Figure 5: Dendrogram represent similarity of micronuclei cell between different groups after 7 and 14 days



a) 26 Chromosomes

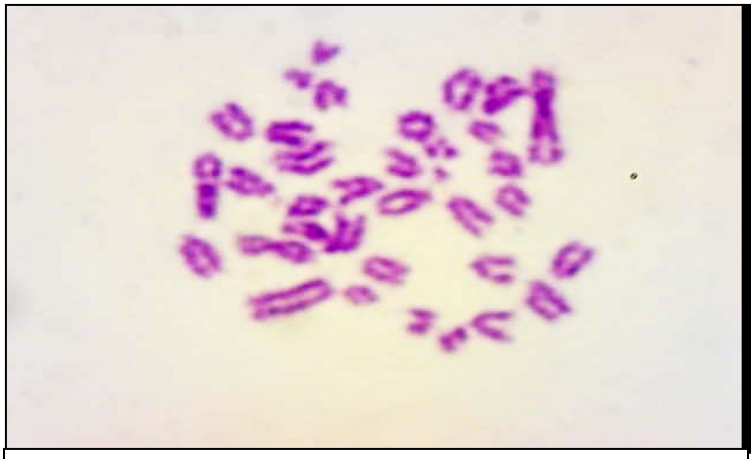


b) 34 Chromosomes

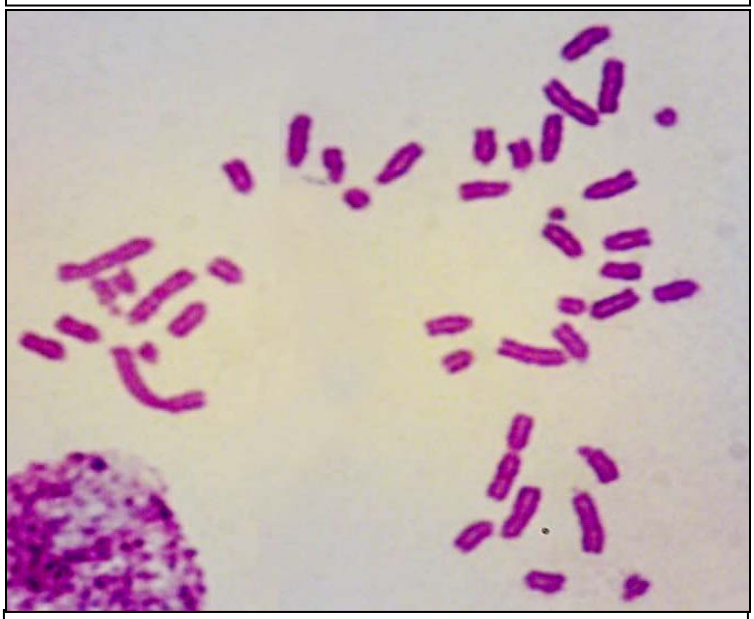


c) 36 Chromosomes

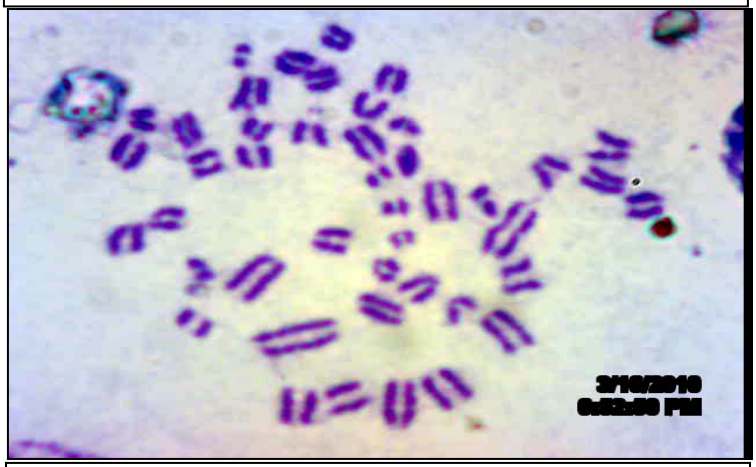
Plate (1): Metaphases showing different chromosome number in Ehrlich ascites cells



a) 40 Chromosomes

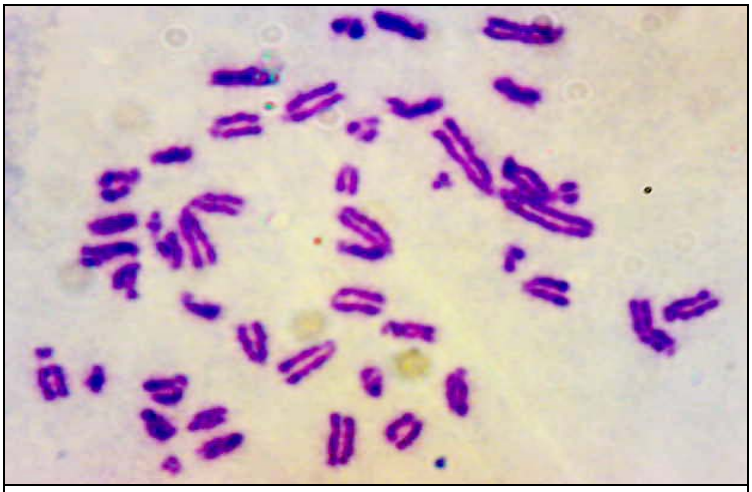


b) 42 Chromosomes

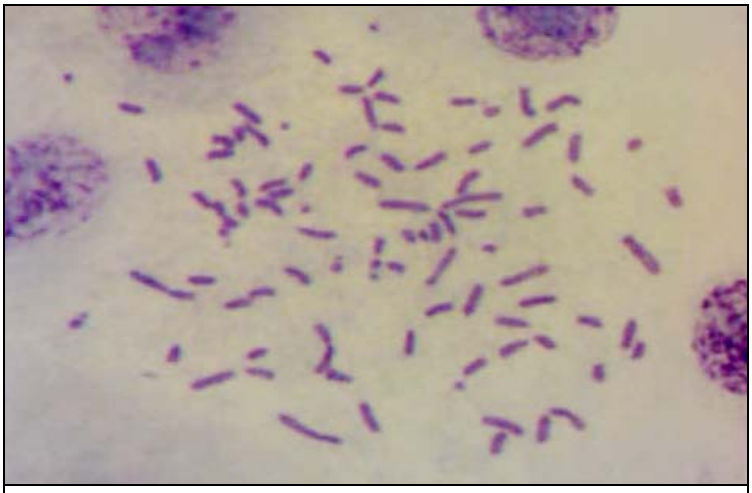


c) 44 Chromosomes

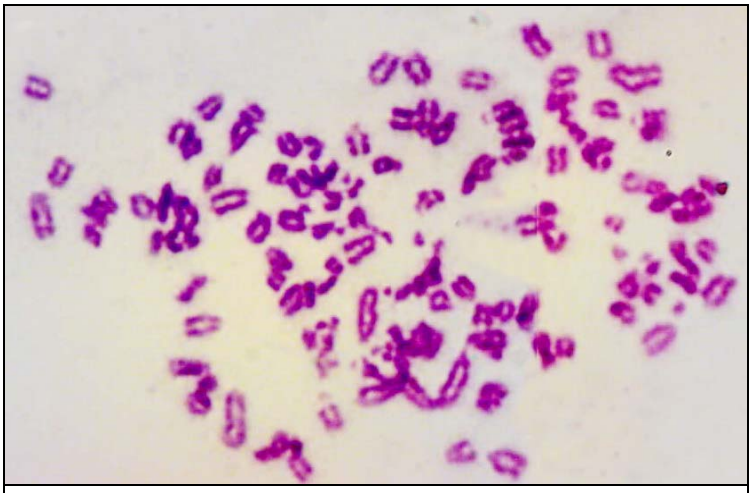
Plate (2): Metaphases showing different chromosome number in Ehrlich ascites cells



a) 46 Chromosomes

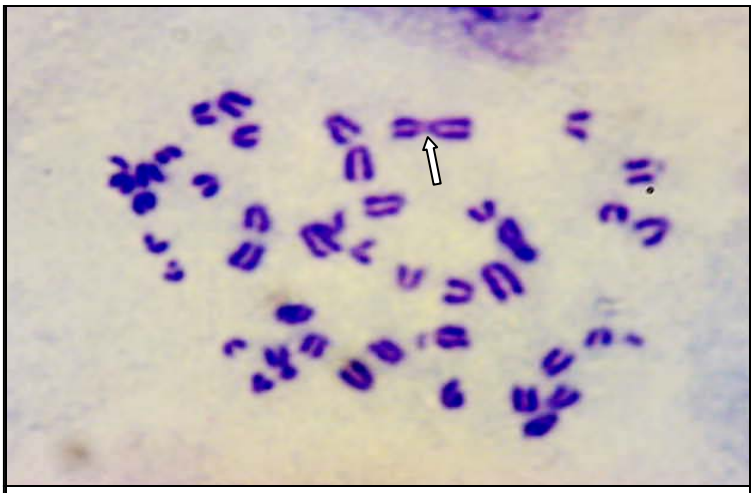


b) 92 Chromosomes

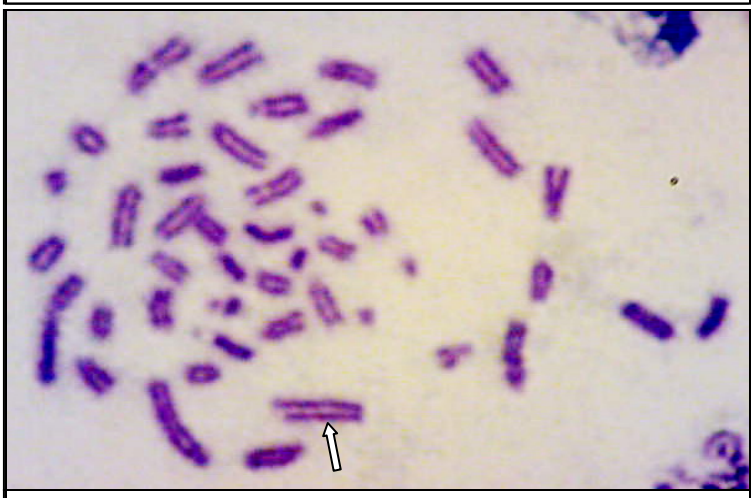


c) 125 Chromosomes

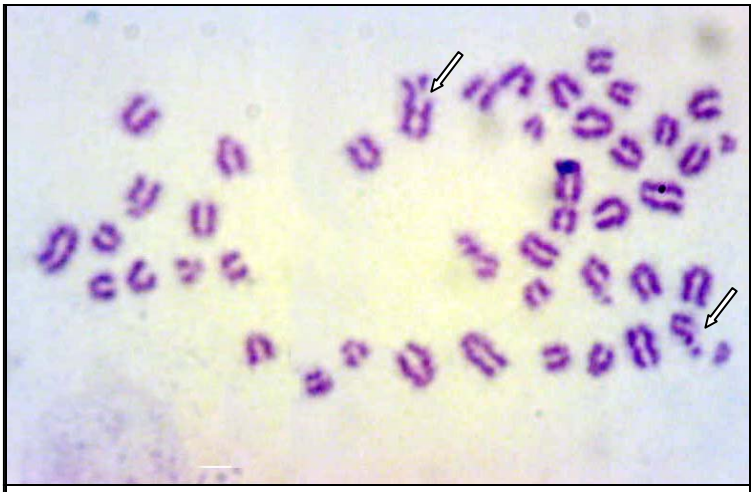
Plate (3): Metaphases showing different chromosome number in Ehrlich ascites cells



a) Centric fusion

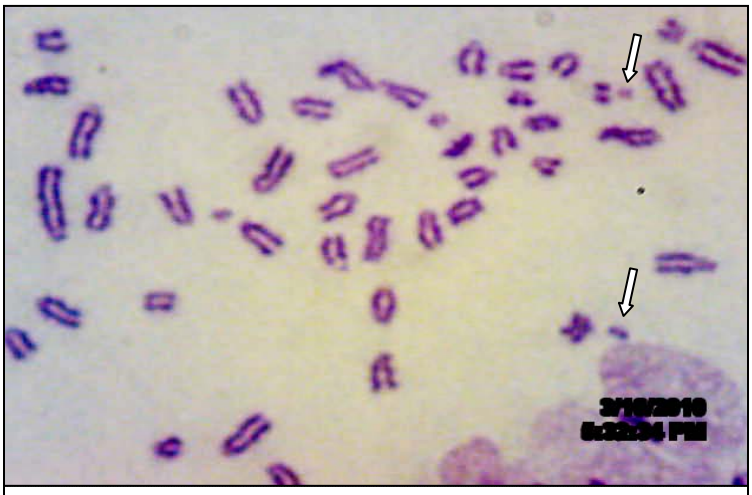


b) Deletion

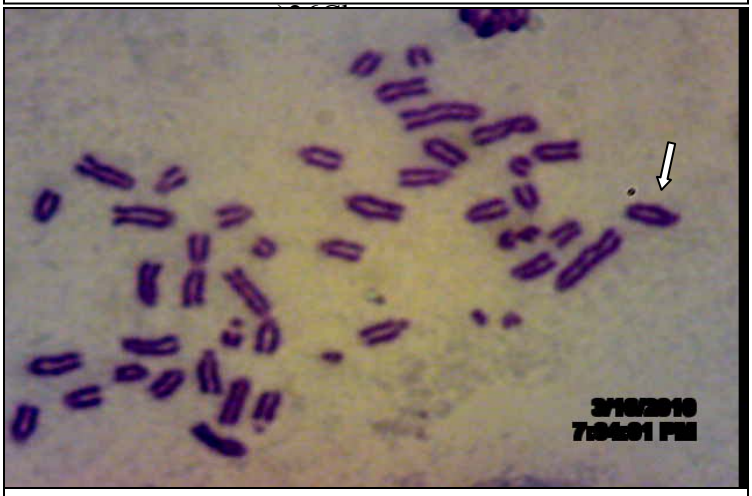


c) Break

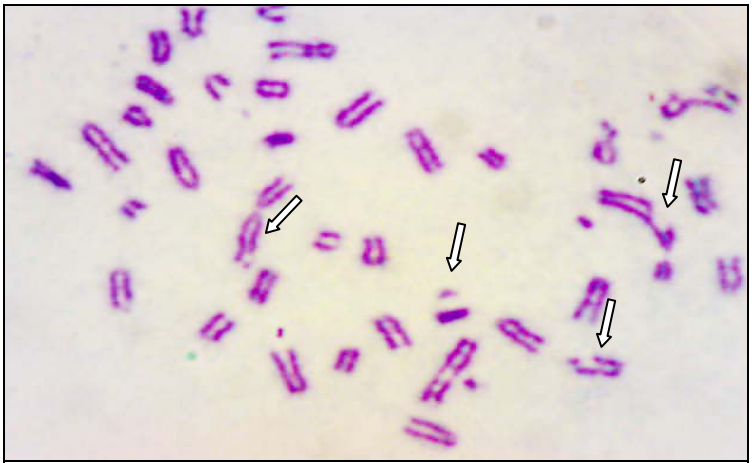
Plate (4): Ehrlich ascites cells metaphases showing, structural chromosomal aberrations



a) Fragment



b) Ring



c) Break, end to end association and fragment

Plate (5): Ehrlich ascites cells metaphases showing, structural chromosomal aberrations

4. Discussion:

Ginger has long been used in traditional medicine as a cure for some diseases including inflammatory diseases (Afzal *et al.,* 2001). Ginger contains active phenolic compounds such as gingerol, paradol and shogoal that have antioxidant (Jeyakumar *et al*., 1999), anti-cancer (Shukla and Singh 2007), anti-inflammatory (Hudson *et al.,* 2006), anti-angiogenesis (Huang *et al*., 2000) and anti-artherosclerotic properties (Coppola and Novo 2007). It has also been shown to down-regulate NF-κB-regulated gene products involved in cellular proliferation and angiogenesis, including IL-8 (Nonn *et al*., 2007).

The present study, showed an increase in frequency of aberrant cell. Various pictures of chromosomal aberrations appeared in Ehrlich ascites cells. These aberrations were manifested in numerical (haploid and polyploidy) and structural aberrations. Treatment with ginger extract reduced aberrant cells. Also ginger extract greatly changed the polyploidy and haploid of tumor cells to diploid (normal morphology). The reduction of abnormalities in tumor cells by the extracts may stimulate cells to divide normally or go to die (through apoptosis) if they cannot remove chromosomal abnormalities. These results confirm these obtained by Vijayapadma, *et al*. (2007), which show that saline extract prepared from ginger extract caused suppression of cell proliferation and marked morphological changes including cell shrinkage and condensation of chromosomes. This is attributed to its anticancer properties due to presence of certain pungent vallinoids, viz. [6]-gingerol and [6]-paradol, as well as some other constituents like shogaols, zingerone etc in its structure (Shukla and Singh 2007). The apoptosis of lung epithelium cancer (A549) cells by aqueous extract of ginger (GAE) is mediated by up regulation of tumor suppressor gene p53 .The morphological change of cells upon GAE treatment has also been demonstrated. Both the structural and functional properties of tubulin and microtubule were lost, as confirmed by both ex vivo and *in vitro* experiments (Choudhury *et al.,* 2010).

The present data revealed that significant increase in the frequency of micronucleated cells in Ehrlich ascites cells. Ginger extract reduce the micronuclei cells in Ehrlich ascites cells. These results confirm these obtained by Nirmala *et al*. (2008) observed increased 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.

Results obtained in the present study demonstrate that ginger extract can reduce the mitotic index in Ehrlich ascites cells. These results may be attributed to gingerdione is one of the components from ginger that has been demonstrated to be an effective anti-tumor agent in human leukemia cells (Hsu, *et al.,* 2005). Also, gingerdione induces G1 arrest in human leukemia HL 60 cells. B-Elemene is a novel anticancer drug, which is extracted from the ginger plant. It triggers apoptosis in non-small-cell lung cancer cells through a mitochondrial release of the cytochrome c-mediated apoptotic pathway (Wang *et al.,* 2005). Also, 6-shogaol exhibited the most potent cytotoxicity against human A549, SK-OV-3, SK-MEL-2, and HCT15 tumor cells (Kim *et al*., 2008). 6-shogaol inhibited proliferation of the transgenic mouse ovarian cancer cell lines. In addition 6-gingerol has two types of antitumor effects: 1) direct colon cancer cell growth suppression, and 2) inhibition of the blood supply of the tumor via angiogenesis (Brown *et al,*. 2009).

5. Conclusion:

Ginger extract may have a chemotherapeutic effect on Ehrlich ascites cells. It is greatly changes tumor cells to diploid normal cells .The use of dietary agents such as ginger may have potential in the treatment and prevention of cancer.

Corresponding author
Zeinab E. Hanafy
Department of Zoology, Faculty of Science (Girls), Al-Azhar University, Cairo, Egypt.
zainabhanafy@gmail.com

6. References:

1. Milner, JA. 2004: Molecular targets for bioactive food components. J Nutr. 134:2492s–2498s.
2. Kuo, P.C.; Damu, A.G.; Cheng, C.Y.; Teng, C. M.;Lee, E.J.; Wu, T.S. 2005: Isolation of a natural antioxidant, dehydrozingerone from Zingiber officinaleand synthesis of its analogues for recognition of effective antioxidant and antityrosinase agents. Archives of pharmacol Research, 28:518-528.
3. Milner J. A. 2001: A historical perspective on garlic and cancer. J Nutr. 131: 1027S–1031S,
4. Keum, Y.S.; Jeong, W.S.and Kong, A.N. 2004: Chemoprevention by isothiocyanates

and their underlying molecular signaling mechanisms. Mutat. Res. 555: 191–202.
5. Musa, D.; Dusiz, N.; Gumushan, H.; Ulakoglu, G. and Bitiren, M. 2004: Antitumor activity of an ethanol extract of Nigella sativa seeds. Biologia Bratialava, 59(6):735-740.
6. Zahran, M. M.; Aboul-Enein and Abo-Ella F.M. 2005: Molecular changes on cancer cells as affected by Willow extracts. Research J.of Agriculture and Biological Sciences, 1(3):284-287.
7. Ahmad, I., Zahin, M., Aqil, F., Hasan, S., Khan,M.S.A. and Owais, M. 2008: Bioactive compounds from Punica granatum, Curcuma longa and Zingiber officinale and their therapeutic potential. Drugs Fut, 33(4):329-239.
8. EL-Merzabani, M.M.; EL- Aaser, A.A.; ELDuweini A.K. and EL-Masry, A.M. 1979b: A bioassay of antimitotic alkaloids of Catharanthus roseus. Med. Plant. Res. 36: 87-90.
9. Duthie, S.J.; Johnson, W. and Dobson, V.L. 1997: The effect of dietary flavonoids on DNA damage (strand breaks and oxidized pyrimidines) and growthin human cells. Mut. Res. 390: 141-151.
10. Gonalez, C.M.; Cuello, M.T. and Larripa, I. 1997:Mitotic arrest and anaphase aberrations induced by vinorelbine in hamster cells *in vitro*. Anti-Cancer-Drugs, 8(5): 529-532.
11. Royman, D.W. and Ruddon, M.D. 1995: Genetic alteration in cancer cells. Cancer Biology, 3:67-88.
12. Pirianov, G.; James, S.Y. and. Colston, K.W. 1998:Vitamin D analogues potentiate TNF-" and ceramide induced apoptosis in human breast cancer cells. British. J. Cancer, 78: 25-71.
13. Filion, M.C.; Shea, R.O.; Collins, J.K. and Phillips, N.C. 1998: Mycobacterial DNA induces apoptosis in tumor cells. Brit. J. Cancer, 78(1): 25-71.
14. Choudhury, D.; Das, A.; Bhattacharya, A.; Chakrabarti,G. 2010: Aqueous extract of ginger shows antiproliferative activity through disruption of microtubule network of cancer cells. Food and Chemical Toxicology, in Press.
15. Jeyakumar, S.M.; Nalini. N. and Menon, V.P.1999: Antioxidant activity of ginger (*Zingiber officinale*) in rats fed a high fat diet.Medical Sciences Research, 27:341–344.
16. Surh, Y.J. 2003: Cancer chemoprevention with dietary phytochemical. Nature Reviews Cancer, *3:768*–780.
17. Xifeng, W.U.; Jian, G. and Spitz, M.R. 2007: Mutagen sensitivity: A genetic predisposition factor for cancer. Cancer Research, 67: 3493-3495.
18. Hung, H.C.; Joshipura, K.J. ; Jiang, R.. ; Hu, F.B; Hunter D. and Smith-Wamer, S.A. *et al.,* 2006:Fruit and vegetable intake and risk of major chronic disease. Journal National Cancer Institute, 96:757-784.
19. Masuda, Y.; Kikuzaki, H.; Hisamoto, M. and Nakatani, N., 2004: Antioxidant properties of gingerol related compounds from ginger. Biofactors, 2: 293–296.
20. Surh, Y.J. 2002: Anti-tumor promoting potential of selected spice ingredients with antioxidative and anti-inflammatory activities: a short review. Food and Chemical Toxicology, 40: 1091–1097.
21. Bode, A. 2003: Ginger is an effective inhibitor of HCT116 human colorectal carcinoma in vivo. Cancer Prevention Research, 26:30-38.
22. Kim, E.C.; Min, J.K.; Kim, T.Y.; Lee, S.J.; Yang, H.O.; Han, S.; Kim, Y.M. and Kwon, Y.G. 2005a: [6]-Gingerol, a pungent ingredient of ginger inhibits angiogenesis in vitro and in vivo. Biochemical and Biophysical Research Communications, 335: 300–308.
23. Vijayapadma, V.; Christie, S.A.D. and Ramkuma, k.M. 2007: :Induction of apoptosis by ginger in HEp-2 cell line is mediated by reactive oxygen species. Basic & clinical pharmacology & toxicology, 100: 302-307.
24. Mothana, R. A.; Lindequist, U.; Gruenert, R and Bednarski, P. J. 2009: Studies of the in vitro anticancer, antimicrobial and antioxidant potentials of selected Yemeni medicinal plants from the island Soqotra. BMC ComplementAlternative Medicine, 9: 7-18.
25. Evans, E.P. 1987: Karyotyping and sexing of gamets , embryos ,fetuses and *in situ* hybridization to chromosomes. In: Mammalian Development. A Practical approach, Non K.M(Ed) pub. IRL, Press Oxford, pp. 93-100.
26. Salamone, M. F.; Heddle, J. A.; Stuart, E. and Katz, A. 1980: Towards and improved

micronucleus test: Studies on 3 model agents mitomycinc, cyclophosphamide and dimethylbenznthracene. Mutation Research, 44: 347-356.

27. Bary-curtis Similarity Inedex: PRIMER Ver,5.0
28. Snedecor, G. W. and Cochran, W.G. 1980: Statistical Methods, 7th ed Iows State unive. Press, Iowa, U.S.A.
29. Afzal, M.; Al-Hadidi, D.; Menon, M.; Pesek, J.and Dhami M.S. 2001: Ginger: an ethnomedical, chemical and pharmacological review. Drug Metabol Drug Interact. 18:159–190.
30. Shukla, Y. and Singh, M. 2007: Cancer preventive properties of ginger: A brief review. Food and Chemical Toxicology, 45:683–690.
31. Hudson, E.A.; Fox, L.H.; Luckett, J.C.A. and Manson, M.M. 2006: Ex vivo cancer chemoprevention research possibilities. Environmental Toxicology and pharmacology, 21:204–14.
32. Huang, S.; DeGuzman, A.; Bucana, C.D.and Fidler, I. J. 2000: Nuclear factor-kappaB activity correlates with growth, angiogenesis, and metastasis of human melanoma cells in nude mice. Clin Cancer Res. 6:2573–2581.
33. Coppola, G. and Novo, S. 2007: Statins and peripheral arterial disease: effects on claudication, disease progression, and prevention of cardiovascular events. Arch Med Res. 38:479–88
34. Nonn, L.; Duong, D. and Peehl, D.M. 2007: Chemopreventive anti-inflammatory activities of curcumin and other phytochemicals mediated by MAP kinase phosphatase-5 in prostate cells. Carcinogenesis, 28:1188–1196.
35. Nirmala, K.; Krishna, T.P and Polasa, K. 2008: Inhibition of induced micronuclei formation in human lymphocytes by ginger. International Journal of Cancer Research, 4:12-19.
36. Hsu, M.H.; Kuo, S.C.; Chen, C.J.; Chung, J.G.; Lai, Y.Y.; Huang, L.J. 2005: 1-(3,4-Dimethoxyphenyl 3,5-dodecenedione (I6) induces G1 arrest and apoptosis in human promyelocytic leukemia HL-60 cells. Leukemia Research, 29: 1399–1406.
37. Wang, G.; Li, X.; Huang, F.; Zhao, J.; Ding, H.; Cunningham, C.; Coad, J.E.; Flynn, D.C.; Reed, E. and Li, Q.Q. 2005:Antitumor effect ofb-elemene in non-small-cell lung cancer cells is mediated via inductionof cell cycle arrest and apoptotic cell death. Cellular and Molecular Life Sciences, 62: 881–893.
38. Kim, J.S.; Lee, S.I.; Park, H.W.; Yang, J.H.; Shin, T.Y.; Kim, Y.C.; Baek, N.I..; Kim, S.H.; Choi, S.U.; Kwon, B.M.; Leem, K.H.; Jung, M.Y. and Kim, D.K. 2008: Cytotoxic components from the dried rhizomes of *Zingiber officinale Roscoe*. Arch. Pharm. Res. 31(4):415- 420.
39. Brown, A.C.; Shah, C.; Liu, J.; Pham, J.T.; Zhang, J.G .and Jadus ,M.R. 2009: Ginger's (*Zingiber officinale Roscoe*) inhibition of rat colonic adenocarcinoma cell proliferation and angiogenesis in vitro. Phytother Res. 23(5):640-645.