

# Cytogenetics Changes on Cancer Cells as Affected by Ginger Extracts

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**Abstract:** A wide variety of phenolic substances derived from spice possess potent antimutagenic and anticarcinogenic activities. Some of the 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  $2.5 \times 10^6$  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 from 26 to 125 with an increase in micronuclei cells and incidence of mitotic index. 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. Numerous constituents of plant foods, including flavonoids (such as quercetin, rutin, and genistein), phenols (such as curcumin, epigallocatechin-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 garlic extracts was investigated (Aboul-Enein, 1986). Soybean seed extracts showed antitumor activity which is due to the presence of trypsin inhibitor (Aboul-Enein *et al.*, 1986). The tumor inhibitors of plant origin depend upon the type of cancer cells and plant species as well as the extract used. Different

plant species (from eight families) growing in Egypt showed anticancer activity (EL-Mrezabani, *et al.*, 1979a). The principles separated from these plants were also studied such as alkaloids (EL-Mrezabani, *et al.*, 1979b; Pokorny, *et al.*, 1983), terpenes (Nozaki, *et al.*, 1990), flavonoids (Hirano, *et al.*, 1994 and Duthie, *et al.*, 1997), or chlorophyll (Sarkar, *et al.*, 1996).

The metaphase of tumor cells was highly arrested by vincristine and vinblastine as alkaloids which have antimetabolic effect (EL-Mrezabani, *et al.*, 1979b). The abnormalities in chromosomes of tumor cells such as sister chromatid change and aberrations (Duthie, *et al.*, 1997; Gonzalez, *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 (Mapara, *et al.*, 1993; Neubauer, *et al.*, 1996; Mehta, *et al.*, 1997; Pirianov, *et al.*, 1998 and Filion, *et al.*, 1998).

Mutagenicity, clastogenicity, cytotoxicity and carcinogenicity are inhibited by antioxidant compounds found in abundance in plants (Hochstein and Atallah, 1988). 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. Tumor promotion is closely linked to inflammation and oxidative stress; so compounds that exhibit anti-inflammatory and/or antioxidant properties could act as anticarcinogenic agent (Masuda *et al.* 2004). Some phenolic substances present in ginger (*Zingiber officinale* Roscoe, Zingiberaceae), generally, possess strong anti-inflammatory and anti-oxidative properties and exert substantial anti-carcinogenic and anti-mutagenic activities (Surh, 2002; Bode, 2003; Kim *et al.*, 2005a and Vijayapadma *et al.*, 2007).

This study aimed 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<sub>3</sub>OH) by Soxhlet extraction for 8 hours. Residue was dried overnight and then extracted with 250 ml water (H<sub>2</sub>O) 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 parent line was supplied through the courtesy of Dr. Gklein, Amsterdam, Holland. The tumor line was maintained in female Swiss albino mice by weekly intraperitoneal injection of 2.5x 10<sup>6</sup> 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:

Eighty 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<sup>6</sup> 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 were subjected to colchicine treatment (0.5 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 prophase and metaphase, was determined.

The micronucleus test: (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 Index. and ANOVA-test according to Snedecor & Cochran (1980) at probability 0.01

### 3. Results and Discussion:

#### Chromosome number aberrations

As shows in Table (1); on day 7 & 14; in Ehrlich group, Ehrlich ascites cells contain different number of chromosomes ranging from 26 to 125 chromosomes [Plate 1,2&3]. The stem cells of this tumor had 46 rod-shaped chromosome [Plate 3 (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 were 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 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 extract significant decrease in the micronucleated cells was observed (Fig. 5).

**Table (1): Distribution of chromosome numbers in Ehrlich ascites cells treated with or without ginger extract after 7 and 14 days**

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+ Ginger 7days	total	4	0	0	4	6	6	8	156	0	16	0
	%	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+ Ginger 14days	total	5	0	0	6	8	0	8	164	0	9	0
	%	2.5	0	0	3	4	0	4	82	0	4.5	0

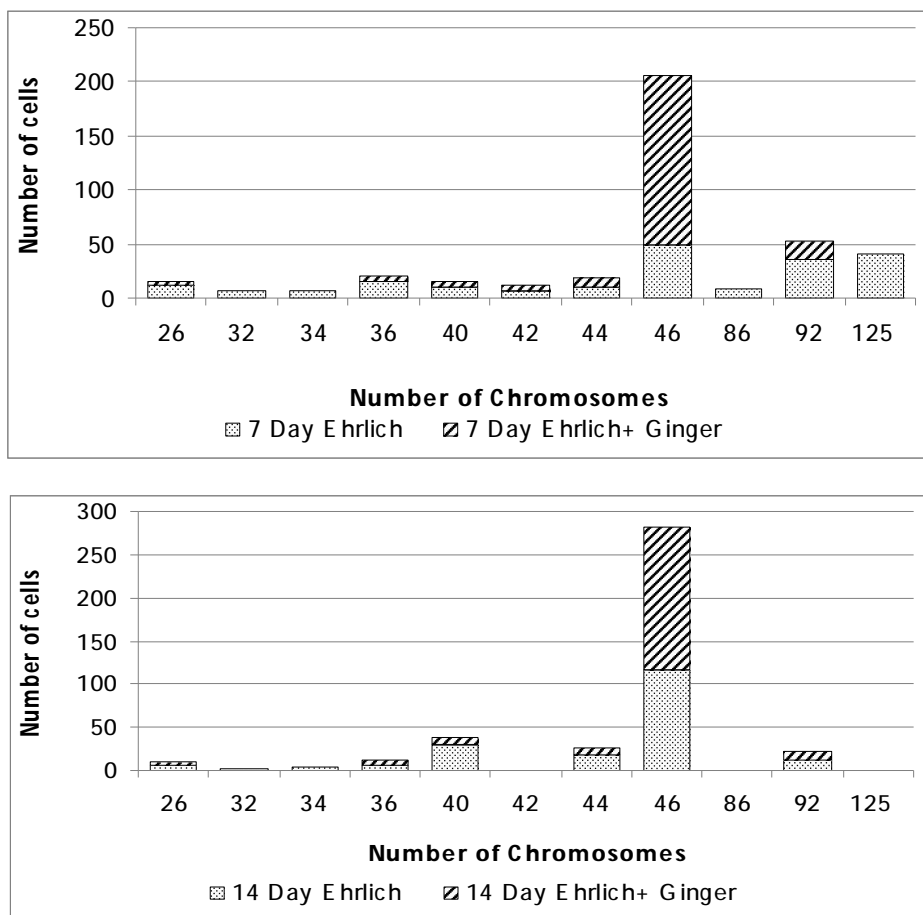


Fig. 1: Distribution of chromosome number in Ehrlich ascites cells treated with ginger extract after 7 and 14days.

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

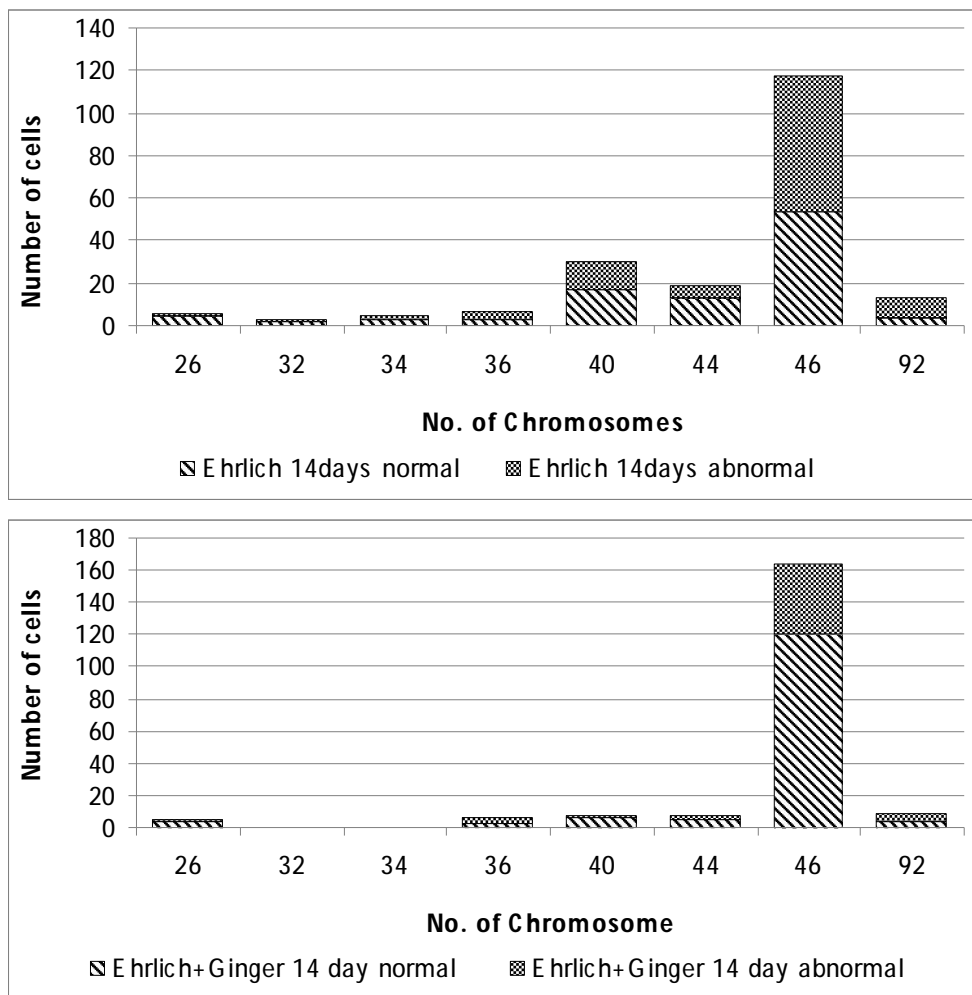
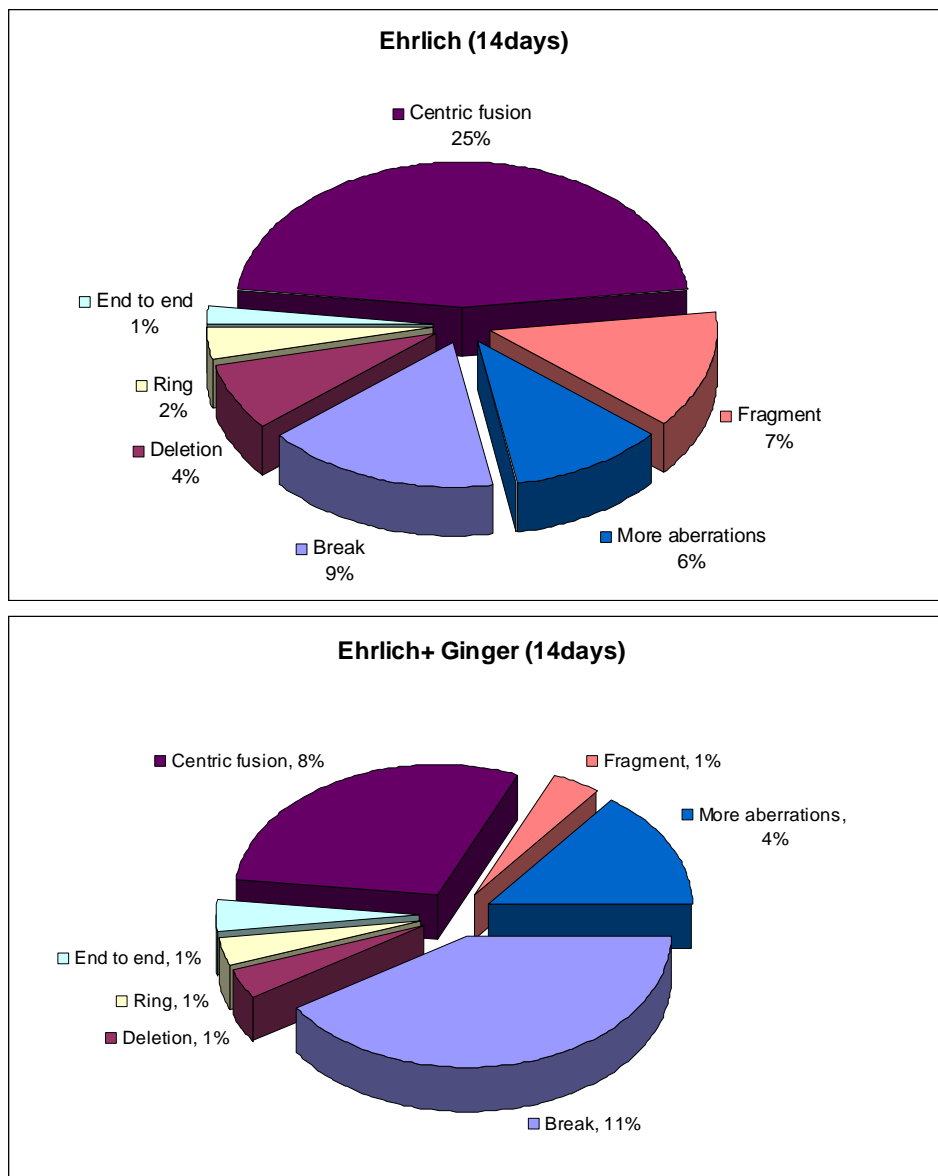


Fig. 2: Distribution of chromosome number in Ehrlich ascites cells (normal and abnormal) treated with or without ginger extract after 14days

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%

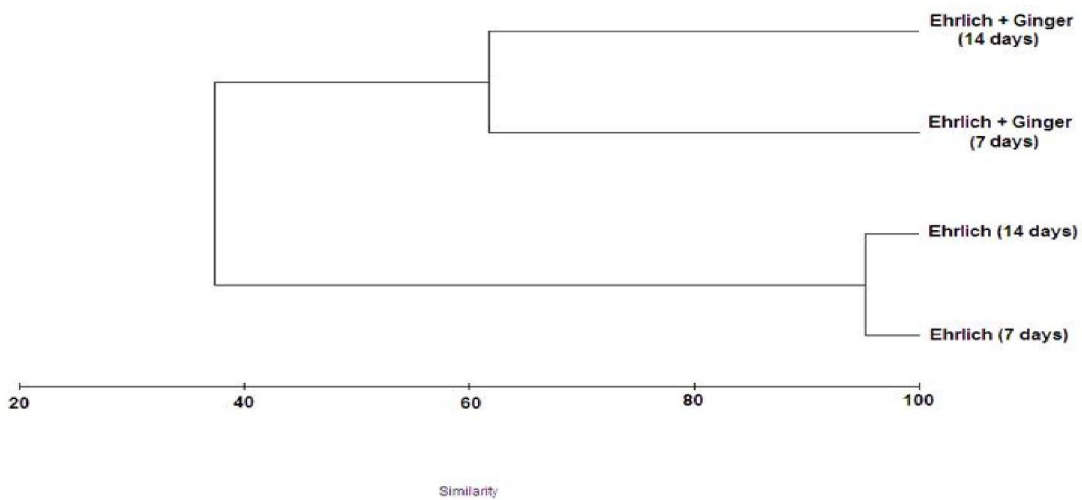


**Fig. 3:** Structural Chromosomal aberration in diploid Ehrlich ascites cells (46chromosomes) treated with or without Ginger extract after 14days

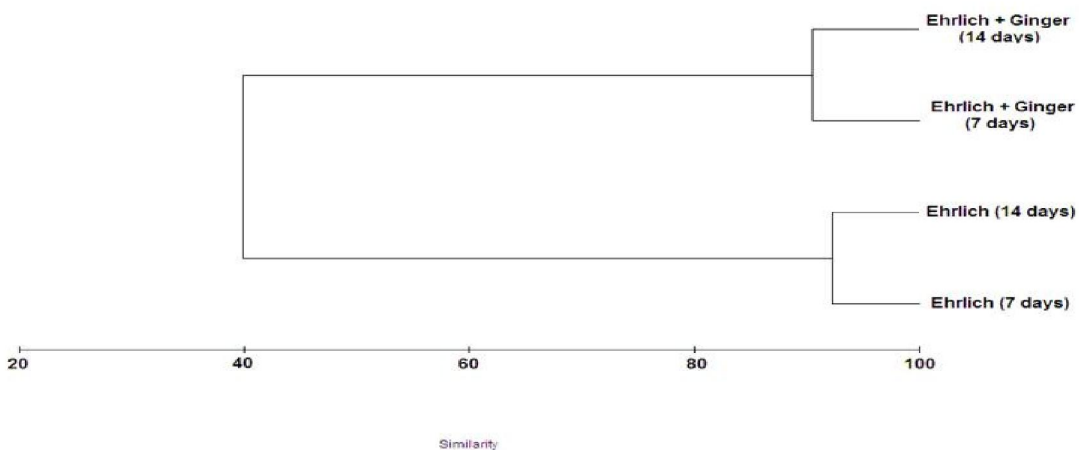
**Table 4:** Mean of mitotic index and micronuclei cell in Ehrlich ascitic tumor cells treated with or without Ginger extract after 7 and 14days.

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

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

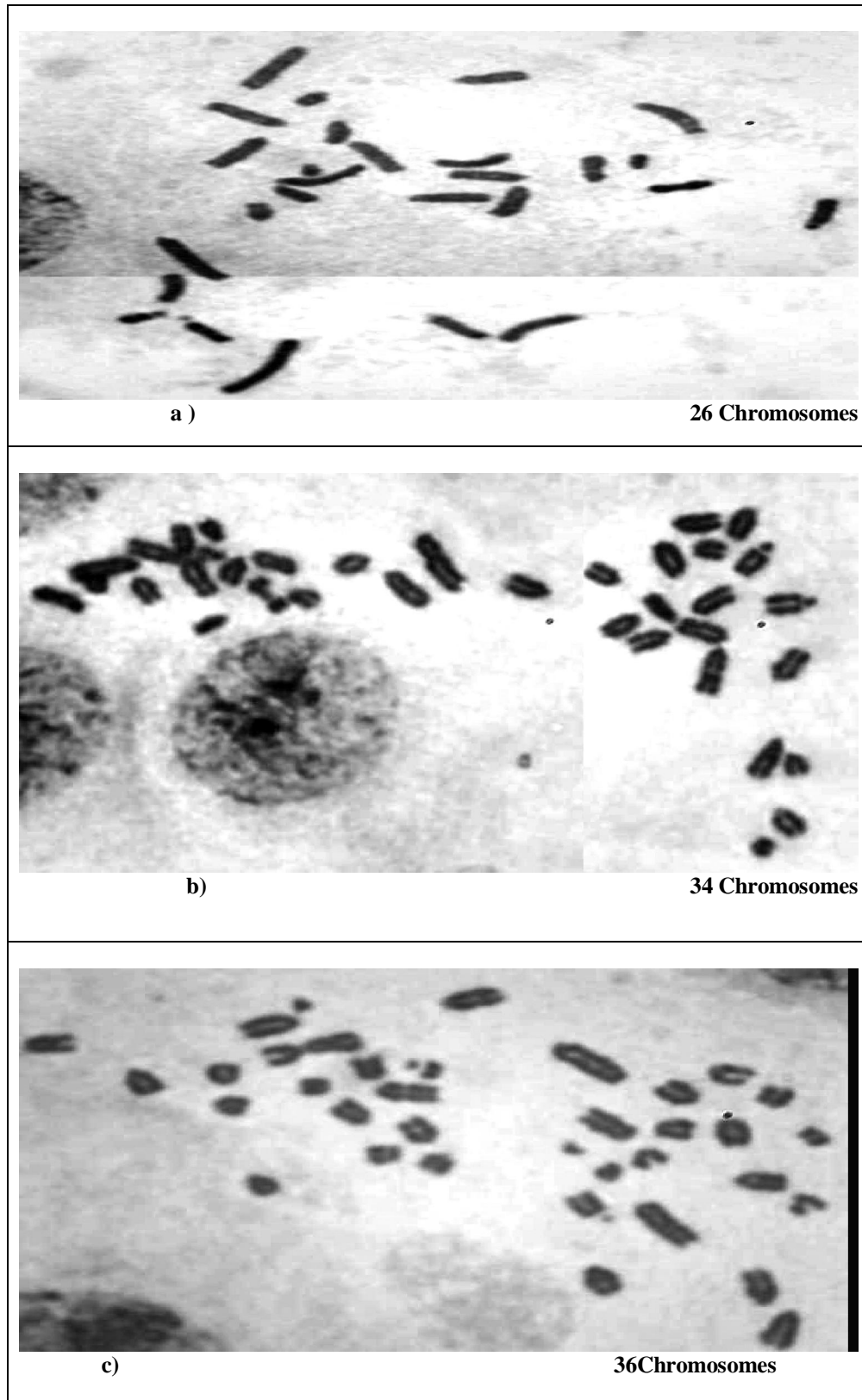


**Figure 4: Dendrogram represent similarity of mitotic index between different groups after 7 and 14 days**



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





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



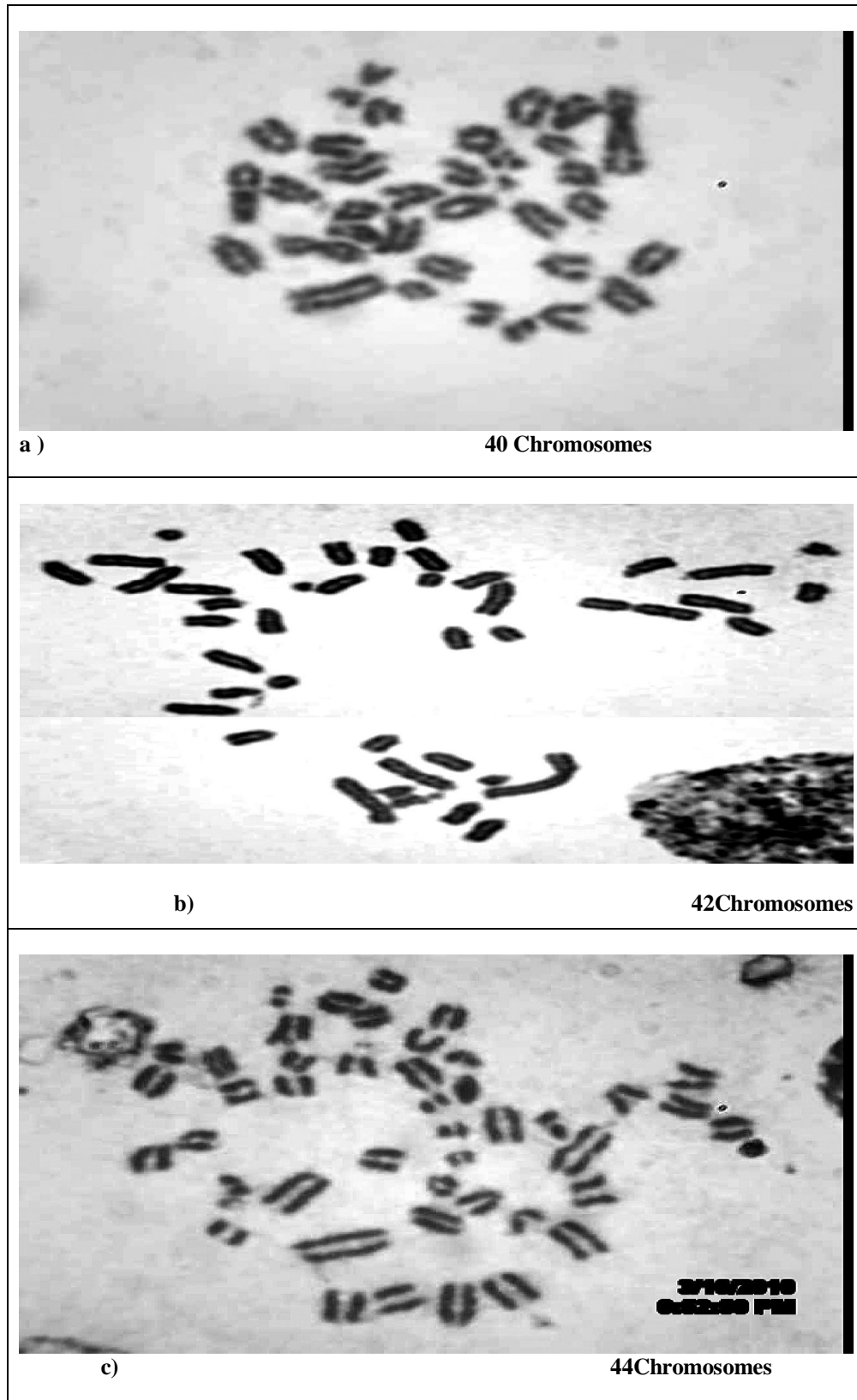


Plate ( 2 ): Metaphases showing different chromosome number in Ehrlich ascites cells

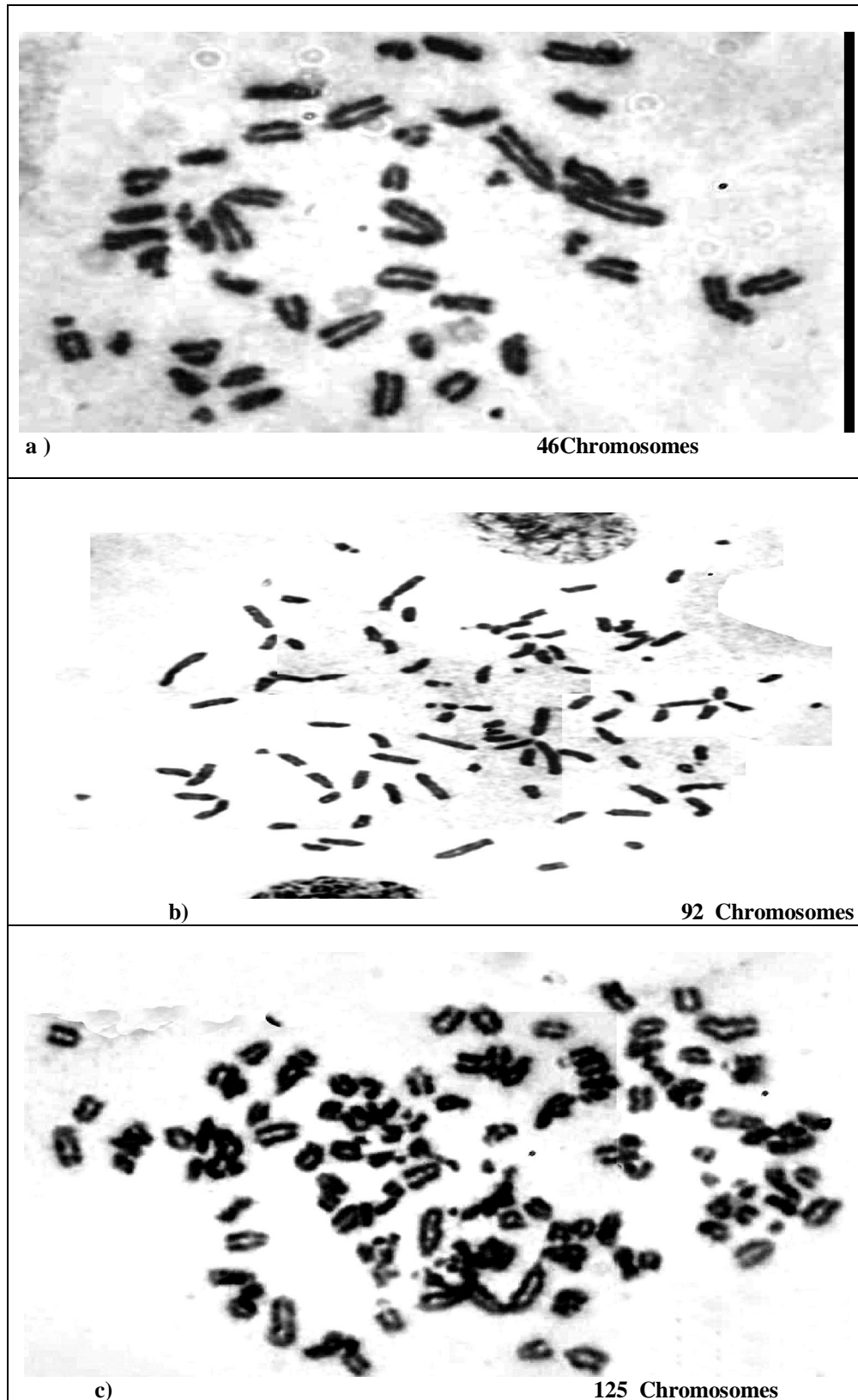


Plate (3): Metaphases showing different chromosome number in Ehrlich ascites cells

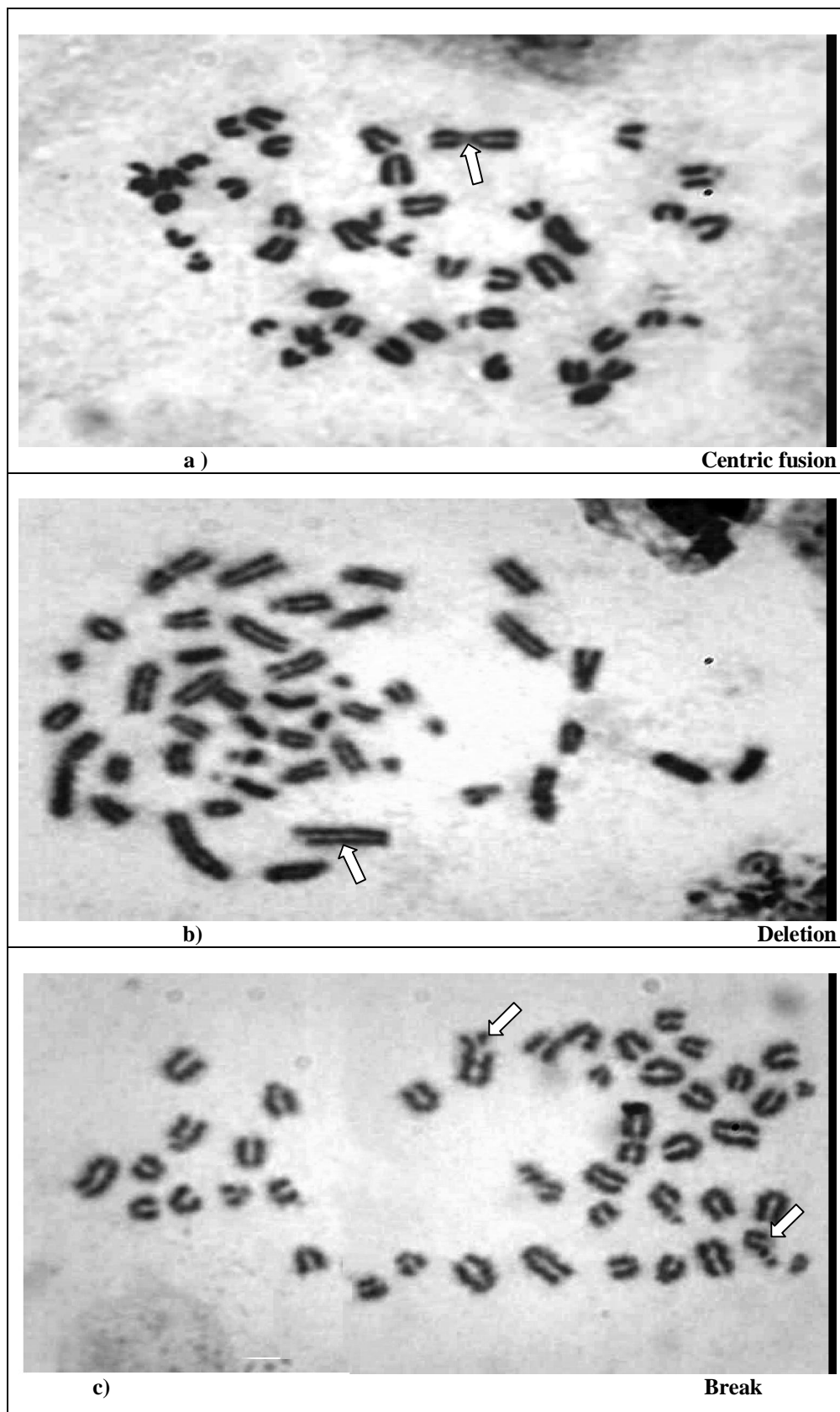


Plate (4): Ehrlich ascites cells metaphases showing, structural chromosomal aberrations

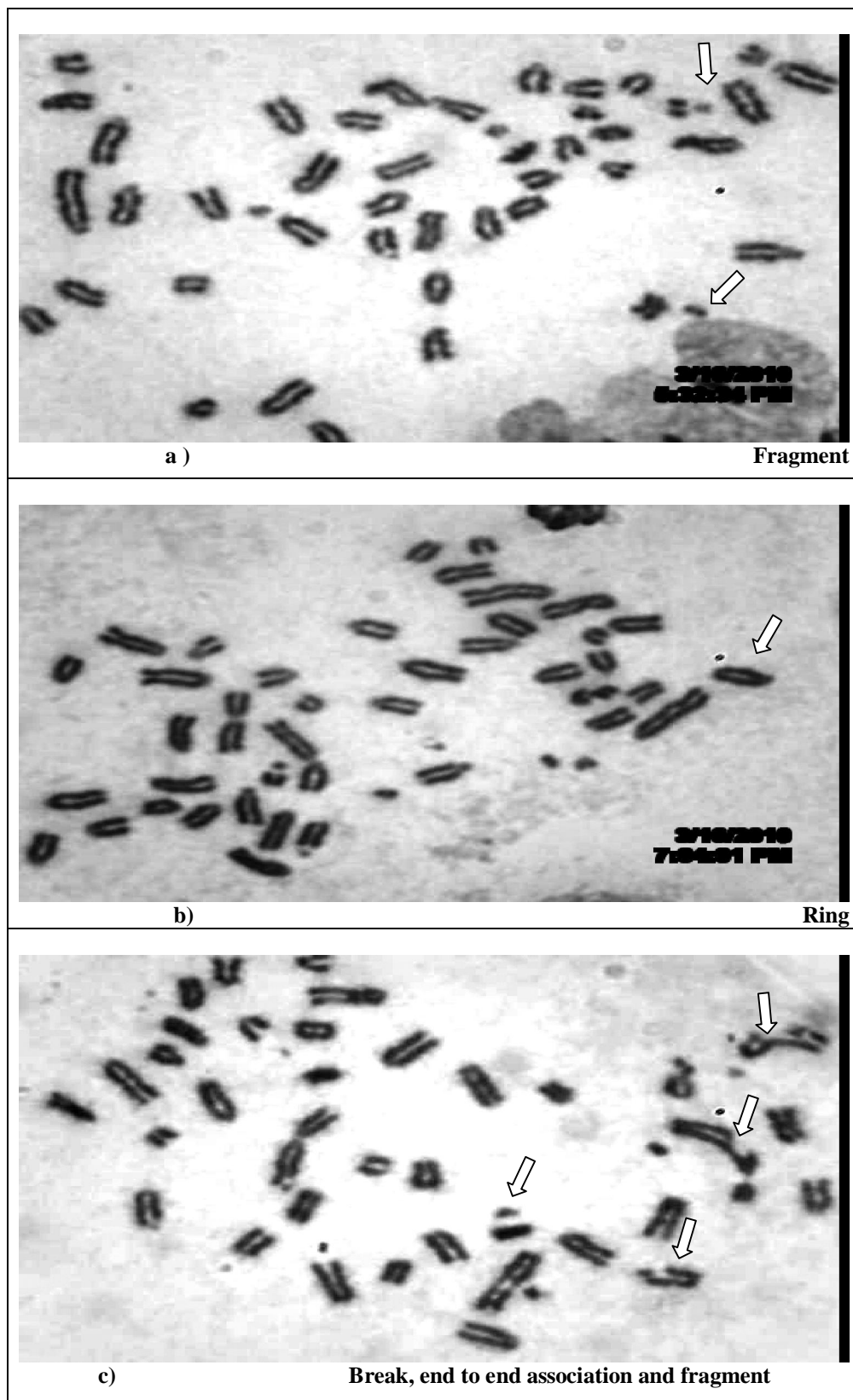


Plate (5): Ehrlich ascites cells metaphases showing, structural chromosomal aberrations



#### 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 shogaol 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-atherosclerotic properties (Coppola and Novo 2007). It has also been shown to down-regulate NF- $\kappa$ B-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 and micronuclei cells. 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 and micronuclei 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 those 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).

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 that 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-Elementene 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.

Color evaluation showed significant differences between Egyptian Gouda cheese related to ripening time, although due to the similar structure the cheeses, the more identical values were found for Water activity and sensory evaluation in Egyptian Gouda cheese.

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