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Cytotoxicity Evaluation Of Aqueous Extracts Of Medicinal Plants On Allium Cepa L.

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Abstract: *In the present investigation cytot-genoxicity of aqueous extracts of ten medicinal plants viz. Syzyum aromaticum (Clove), Oxalis corniculata, Solanum nigrum, Azadirachta indica, Bauhinia variegate, Coriander sativum, Anisomeles indica, Terminallia bellerica, Ocimum sanctum and Citrus sinensis was evaluated on newly growing Allium cepa roots at three different concentrations. The results revealed that the aqueous leaf extracts caused a significant inhibition in the number and growth rate of Allium cepa roots in comparison to negative and positive control. The aqueous leaf extracts caused strong inhibition of dividing cells and mitotic index (MI) values. The chromosomal breaks showed the clastogenic effect of aqueous leaf extracts. The occurrence of chromosome fragments showed statistically significant differences at various concentrations. The stickiness of metaphase chromosomes and polar deviations were observed. A statistically significant increase in total aberrant cells (P < .05) was observed in comparison to the negative control. The aqueous leaf extracts showed the strongest genotoxic effects in the root meristem cells. The appearance of sticky metaphase chromosomes reinforces the hypothesis of the toxic effect of leaf extracts. Metaphases with sticky chromosome loses their normal appearance, and with a sticky "surface," they cause chromosome agglomeration.*

Key Words: *Medicinal plants, Cyto-genotoxicity, Allium cepa, Aqueous extract, Mitotic Index*

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I. Introduction

Medicinal plants have been used in folk medicine for longer lime. However, some plants cause adverse effects, and have the capacity to interact with other medications [1] (Zink and Chaffin, 1998). It has been postulated that the plants or plant products used in medicine have low toxicity. In general green plants are a primary source of antimutagens as well as natural toxic agents [2] (Plewa and Wagner, 1993). However, it has been pointed out that some constituents of have been shown to carcinogenic, teratogenic and mutagenic and their use has been correlated with high rate of tumor formation in some human populations [3, 4, 5, 6, 7, 8, 9] (Moody *et al*., 1999; Gadano *et al*., 2000; Gadano *et al*., 2002; Gadano *et al*., 2006; Effrain *et al*., 2001; Teixera *et al*., 2003; Paes-Leme *et al*., 2005). Many medicinal plants contain cytotoxic and genotoxic substances. Many plants used as food or in traditional medicine have mutagenic effects and cytotoxic and genotoxic effects which have been proved *in vitro* as well as *in vivo* assays [10, 11, 12, 13] (Higashimoto *et al*., 1993; Schmmer *et al*., 1994; Kassie *et al*., 1996; Askincelik and Aslanturk, 2007).

Allium cepa has been most frequently used plant material for cytoxicity and genotoxicity assay [14, 15, 16, 17, 18, 19] (Fiskesjo, 1985; Rank, 2003; Nabeel *et al*., 2008; Algarni, 2018; Li *et al*., 2010, 2015). The bioassay on *Allium cepa* is based on cytotoxicity and genotoxicity using chromosomes. The cyto-genotoxicity assay of aqueous extracts of ten medicinal plants viz. *Syzyum aromaticum* (Clove), *Oxalis corniculata, Solanum nigrum, Azadirachta indica, Bauhinia variegate, Coriander sativum, Anisomeles indica, Terminallia bellerica, Ocimum sanctum* and *Citrus sinensis* on growing root meristems of *Allium cepa* has not been studied and hence the present investigation has been undertaken.

II. Materials and Methods

In the present investigation cytotoxicity of aqueous extracts of ten medicinal plants was evaluated on newly growing *Allium cepa* roots at three different concentrations viz. 2.5 mg/ml, 5.0 mg/ml and 10.0 mg/ml. The leaves of each of the ten medicinal plants viz. *Syzyum aromaticum* (Clove), *Oxalis corniculata, Solanum nigrum, Azadirachta indica, Bauhinia variegate, Coriander sativum, Anisomeles indica, Terminallia bellerica, Ocimum sanctum* and *Citrus sinensis* were rinsed separately with water, dried in an oven at 55^0C for 24h and subsequently ground to a fine powder. The powder of each plant was placed in small plastic bags and stored at

4^0C until use. The extract was prepared by boiling 20 g powdered of each plant in 200 ml of distilled water for (10% stock solution) for 5 min and, cooled to room temperature for 10 min. Thus 10% stock solution was prepared separately for each of the ten medicinal plants.

Stock solution was the diluted with distilled water to prepare three different concentrations viz. 2.5 mg/ml, 5mg/ml, and 10 mg/ml. The cytotoxicity was determined on the basis of physicochemical parameters, root length, root growth, colour, mitotic index and chromosomal aberrations. The mitotic index (MI) was calculated as the ratio between the number of mitotic cells and the total number of cells scored and expressed as percentage and chromatin aberrations (stickiness, breaks and polar deviation) were used as endpoints for determination of cytogenetic effects and micronuclei (MNC) were scored in interphase cells per 1000 cells (‰ MNC) following the method as suggested by [20] Pevlica *et al*., 2000.

Toxicity assay on *Allium cepa*: Small bulbs of about 1.5–2.0 cm in diameter of onion ($2n = 16$) were purchased from a local market and the outer dry scales and the dry bottom plate were removed without destroying the root primordia. For each extract sample, a series of five bulbs were placed in tap water (pH 7.3) for 48 h and then onion roots were treated with the aqueous extracts of leaves of ten medicinal plants separately at concentrations 2.5mg/ml, 5 mg/ml, and 10mg/ml concentrations. The test tubes were kept at 22 ± 2^0C and the test samples were changed daily. The newly grown root tips were then cut from each bulb and examined visible morphological abnormalities. The bulbs with satisfactory root lengths (2– 2.5 cm) were used in the study. Tap water (pH 7.3) was used as a negative control as suggested by [21, 22] Fiskesjo, 1993 and Fiskesjo, 1997, and Ethyl methanesulfonate (EMS, $2 \text{ X } 10^{-2}$ M) used as a positive control mutagen. EMS is a mutagen used in wide range of biological assay [23, 24, 25] (Sega, 1984; Platzek, 1995; Bokel, 2008). EMS induces DNA damage by a direct mechanism, acting at various sites as a monofunctional ethylating agent of nucleotides [26] (Muller *et al*., 2009). After 24 h of exposure, root tips were removed from the bulbs, fixed in 3: 1 (v/v) ethanol: glacial acetic acid and stored overnight at 4^0C. The root tips were then placed in 70% (v/v) aqueous alcohol and refrigerated until used. An average of five slides was made for each bulb using five root tips which hydrolyzed in 1N hydrochloric acid (HCl) for 3 min and microscope slides were prepared by squashing the stained root tips in 2% (w/v) acetic orcein. The slides were then examined under high power of light microscope. The mitotic index (MI), Micronucleus and Aberrant cells were measured by following equations:

$$\text{Mitotic Index (MI)} = \frac{\text{Number of cells in mitosis}}{\text{Total number of cells}} \text{ X } 100$$

$$\% \text{ Micronucleus} = \frac{\text{Total number of micronuclei}}{\text{Total number of cells in interphase}} \text{X}100$$

$$\% \text{ Aberrant cells} = \frac{\text{Total number of aberration}}{\text{Total number of cells}} \text{X}100$$

The root lengths were measured and used as an index of general toxicity after 72 hours of exposure of extract. The results for mitotic index and root length are expressed as percent of the negative and positive control. The visible morphological abnormalities such as root consistency, colour, presence of swelling (c-tumors), hooks in roots etc. was also observed. The data were examined by measuring SD and one way analysis of variance (ANOVA). Differences between corresponding controls and exposure treatments were considered statistically significant at $P < .05$. The results of cytotoxicity have been presented in Table-1, 2 and 3; Fig-3 a-d and 4 a-q.

III. Results

Table-1: Cytotoxicity of medicinal plant extracts on growing roots of Onion (*Allium cepa*)

Treatment group	Concentration of extract in mg/ml	Average root number ±SD	Average root length (cm) ±SD
Negative control	**Tap water**	**40.70 ±3.05**	**4.67 ±0.73**
Positive control (EMS)	**2 X 10^{-2}M**	**38.50 ±3.75***	**3.75 ±0.51***
Syzium aromaticum	**2.5**	**33.40 ±2.71***	**3.45 ±0.61***
	5.0	**28.50 ±3.61***	**3.25 ±0.45***
	10.0	**21.50 ±4.35***	**2.85 ±0.51***
Oxalis corniculata	**2.5**	**35.50±3.25***	**3.15±0.41***
	5.0	**26.50±4.31***	**2.75±0.62***
	10.0	**24.40±4.32***	**2.25±0.41***

Solanum nigrum	**2.5**	**35.70±4.05***	**3.21±0.23***
	5.0	**26.30±3.37***	**2.71±0.24***
	10.0	**24.10±2.45***	**2.21±0.26***
Azadirachta indica	**2.5**	**32.50±5.05***	**3.25±0.21***
	5.0	**26.50±3.21***	**3.07±0.26***
	10.0	**20.60±2.61***	**1.76±0.51***
Bauhinia variegata	**2.5**	**36.30±4.41***	**3.17±0.41***
	5.0	**27.10±4.07***	**2.71±0.31***
	10.0	**24.00±3.05***	**2.27±0.41***
Coriander sativum	**2.5**	**36.70±2.31***	**3.18±0.21***
	5.0	**26.80±3.21***	**2.75±0.61***
	10.0	**24.50±2.06***	**2.76±0.53***
Anisomeles indica	**2.5**	**36.60±4.45***	**3.16±0.61***
	5.0	**26.50±4.37***	**2.71±0.28***
	10.0	**24.30±2.38***	**2.70±0.27***
Terminalia bellerica	**2.5**	**32.40±3.51***	**3.31±0.31***
	5.0	**25.30±4.05***	**3.02±0.43***
	10.0	**20.40±2.37***	**1.65±0.15***
Ocimum sanctum	**2.5**	**36.40±5.05***	**3.12±0.23***
	5.0	**26.30±3.19***	**2.75±0.16***
	10.0	**24.50±2.27***	**2.71±0.21***
Citrus sinensis	**2.5**	**36.70±4.38***	**3.15±0.31***
	5.0	**26.50±3.35***	**2.65±0.21***
	10.0	**23.40±2.36***	**2.61±0.17***

***P* < .05**

Table-2: Dividing and total cells counted under microscopic observations and mitotic values in control and treatment concentrations

Treatment group	Concentration of extract in mg/ml	Total cells	Dividing cells	MI (%) ±SE
Negative control	**Tap water**	**25000**	**1790**	**7.01 ± 1.18**
Positive control (EMS)	**2 X 10^{-2}M**	**25000**	**585**	**2.29 ± 0.85***
Syzium aromaticum	**2.5**	**25000**	**950**	**3.72 ± 0.75***
	5.0	**25000**	**620**	**2.43 ± 0.35***
	10.0	**25000**	**23**	**0.090 ± .05***
Oxalis corniculata	**2.5**	**25000**	**960**	**3.76 ± 0.65***
	5.0	**25000**	**625**	**2.45 ± 0.63***
	10.0	**25000**	**25**	**0.098 ±0.03***
Solanum nigrum	**2.5**	**25000**	**965**	**3.78 ± 0.63***
	5.0	**25000**	**635**	**2.49 ± 0.25***
	10.0	**25000**	**28**	**0.010± 0.05***
Azadirachta indica	**2.5**	**25000**	**921**	**3.61± 0.85***
	5.0	**25000**	**575**	**2.25 ± 0.45***
	10.0	**25000**	**22**	**0.086 ±0.03***
Bauhinia variegata	**2.5**	**25000**	**971**	**3.80 ± 0.75***
	5.0	**25000**	**637**	**2.49 ± 0.46***
	10.0	**25000**	**27**	**0.010 ± .02***
Coriander sativum	**2.5**	**25000**	**975**	**3.82 ± 0.55***
	5.0	**25000**	**635**	**2.49 ± 0.45***
	10.0	**25000**	**26**	**0.010 ± .04***
Anisomeles indica	**2.5**	**25000**	**965**	**3.78 ± 0.91***
	5.0	**25000**	**632**	**2.47± 0.75***
	10.0	**25000**	**27**	**0.010 ±0.03***
Terminalia bellerica	**2.5**	**25000**	**920**	**3.60 ± 0.62***
	5.0	**25000**	**573**	**2.24 ± 0.35***
	10.0	**25000**	**21**	**0.082± 0.06***
Ocimum sanctum	**2.5**	**25000**	**923**	**3.61± 0.55***
	5.0	**25000**	**576**	**2.25 ± 0.15***
	10.0	**25000**	**23**	**0.090 ± .05***
Citrus sinensis	**2.5**	**25000**	**965**	**3.78 ± 0.41***
	5.0	**25000**	**581**	**2.27± 0.17***
	10.0	**25000**	**24**	**0.094 ±0.03***

***P* < .05**

Table-3: Chromosomal and mitotic aberrations in the root meristematic cells of *Allium cepa* after treatment of extracts of medicinal plants

Treatment group	Concentration of extract in mg/ml	Chromosome breaks (%) ±SD	Stickness (%) ±SD	Polar deviation (%) ±SD	Aberrant cells (%) ±SD	MNC ($^0/_{00}$) ±SD
Negative control	Tap water	-	0.70 ±0.05	8.75 ±1.91*	8.45 ±1.87*	0.30 ±0.19*
Positive control (EMS)	2 X 10^{-2}M	-	32.25 ±3.65*	9.65 ±2.17	41.75±4.13	0.71 ±0.19*
Syzium aromaticum	2.5	7.25 ±3.15*	17.35 ±3.25*	6.15 ±1.33*	31.57 ± 2.15	0.66 ±0.15*
	5.0	0.94 ±0.06	28.65 ±3.16	11.15 ±1.34*	40.85 ±3.14	0.51 ±0.14
	10.0	-	9.67 ±1.41	9.65 ±1.67*	20.35 ±2.25	0.06 ±0.02*
Oxalis corniculata	2.5	7.26 ±3.17*	17.37±3.35*	6.25±1.33*	30.67± 2.27*	0.67±0.13*
	5.0	0.95 ±0.07	28.71±2.26*	11.17±1.33*	40.25± 3.16*	0.52±0.11*
	10.0	-	9.65±1.25*	9.65±1.35*	20.45± 2.18*	0.06±0.03*
Solanum nigrum	2.5	7.25 ±2.45*	17.35±3.36*	6.31±1.31*	30.65± 3.05*	0.66±0.15*
	5.0	0.96 ±0.05	28.67±2.26*	11.65±1.37*	39.85± 2.25*	0.49±0.14*
	10.0	-	9.64±1.21*	9.60±1.23*	19.67± 2.13*	0.05±0.02*
Azadirachta indica	2.5	7.27 ± 2.26*	17.65±3.41*	6.37±1.21*	30.67± 2.26*	0.67±0.15*
	5.0	0.95 ±0.07	28.65±2.15*	11.67±1.34*	41.85± 3.16*	0.49±0.12*
	10.0	-	9.64±1.23*	9.66±1.31*	20.75± 2.16*	0.05±0.03*
Bauhinia variegata	2.5	7.25 ±2.25*	17.25±2.26*	6.32±1.23*	30.63± 2.15*	0.73±0.15*
	5.0	0.93 ±0.05	28.74±3.17*	11.64±1.13*	39.35± 3.17*	0.51±0.15
	10.0	-	9.65±1.06*	9.65±1.34*	19.63± 1.16*	0.09±0.03*
Coriander sativum	2.5	7.25 ±1.78*	16.35±2.21*	6.36±1.23*	30.60± 2.15*	0.62±0.15*
	5.0	0.92 ±0.06	28.73±3.41*	11.63±1.17*	39.55± 2.14*	0.55±0.13*
	10.0	-	9.64±1.25*	9.59±1.36*	19.55± 2.18*	0.07±0.04*
Anisomeles indica	2.5	7.28 ±1.75*	17.34±2.16*	6.35±1.34*	30.62± 2.17	0.65±0.17*
	5.0	0.96 ±0.08	28.75±2.16*	11.62±1.23*	39.65± 3.18*	0.53±0.12*
	10.0	-	9.67±1.24*	9.58±1.23*	19.45± 2.12*	0.08±0.03*
Terminalia bellerica	2.5	7.24 ±1.65*	16.32±2.23*	6.33±1.03*	30.66± 2.24*	0.71±0.16*
	5.0	0.91 ±0.04	28.45±3.17*	11.55±1.27*	40.65± 3.16*	0.54±0.13*
	10.0	-	9.62±1.22*	9.60±1.21*	20.65± 2.12*	0.09±0.05*
Ocimum sanctum	2.5	7.23 ±2.15*	17.31±3.05*	6.31±1.16*	30.64± 2.17*	0.64±0.12*
	5.0	0.94 ±0.05	28.51±3.06*	11.45±2.24*	39.65± 3.17*	0.55±0.11*
	10.0	-	9.63±1.12*	9.64±1.18*	19.62± 2.13*	0.09±0.05*
Citrus sinensis	2.5	7.27 ±2.35	17.41±2.31*	6.34±1.07*	30.65± 2.26*	0.65±0.15*
	5.0	0.93 ±0.07	28.61±3.15*	11.67±1.41*	39.75± 2.12*	0.56±0.15*
	10.0	-	9.64±1.60*	9.62±1.05*	19.61± 2.12*	0.08±0.03*

***P* < .05**

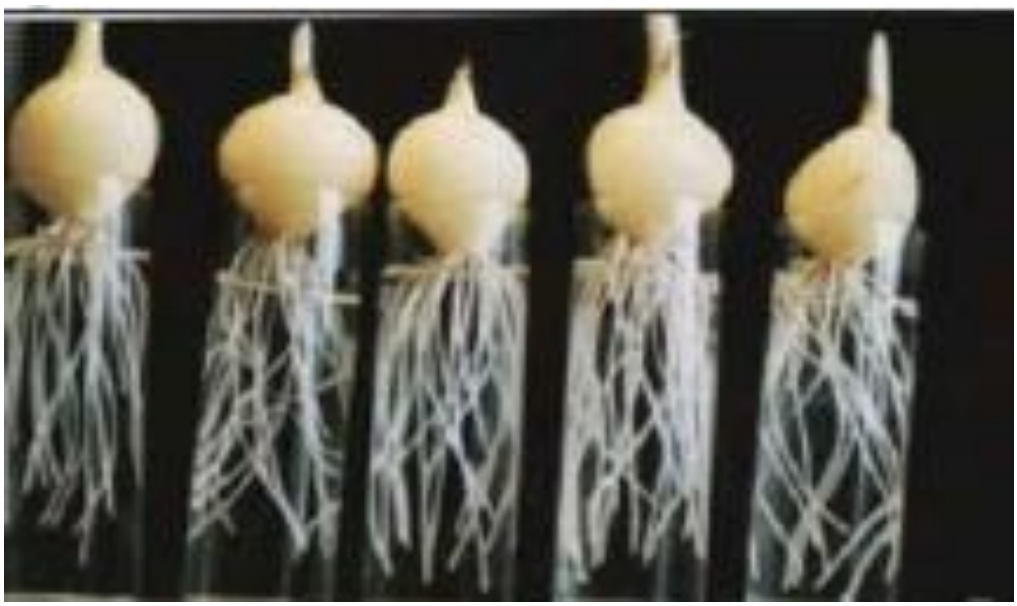

Figure-3a: General toxicity in *Allium cepa*, Length of root in cm in Negative Control

Figure-3b: General toxicity in *Allium cepa* root, Length of root in cm in Positive control

Figure-3c: General toxicity in *Allium cepa* root, Length of root in cm in 2.5mg/ml treated extract

Figure-3d: General toxicity in *Allium cepa* root, Length of root in cm in 10 mg/ml treated extract

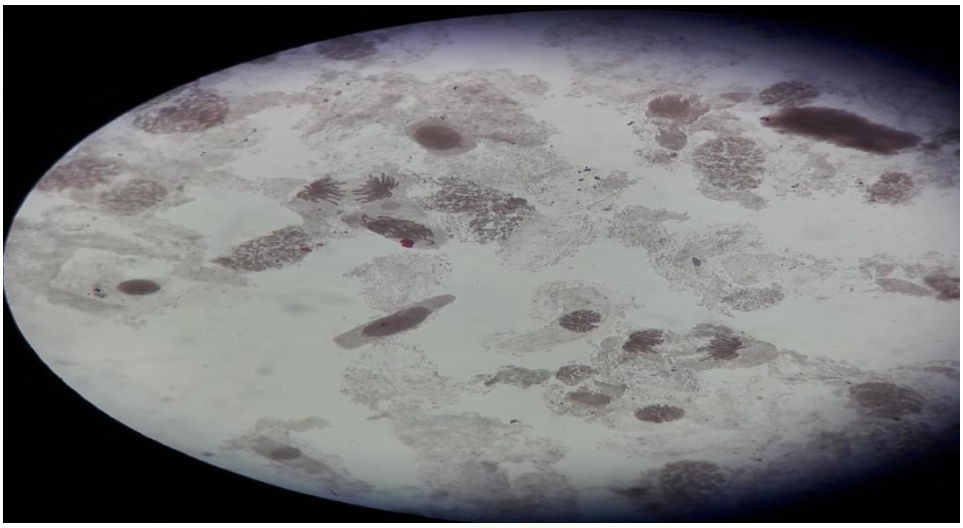

Figure-4a: Chromosomal fragments

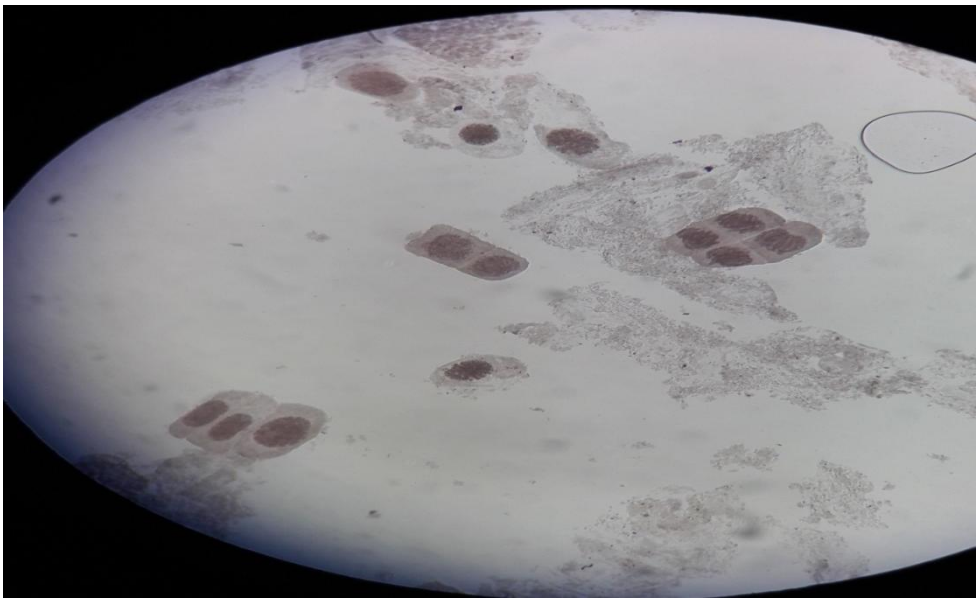

Figure-4b: Chromosomal fragments

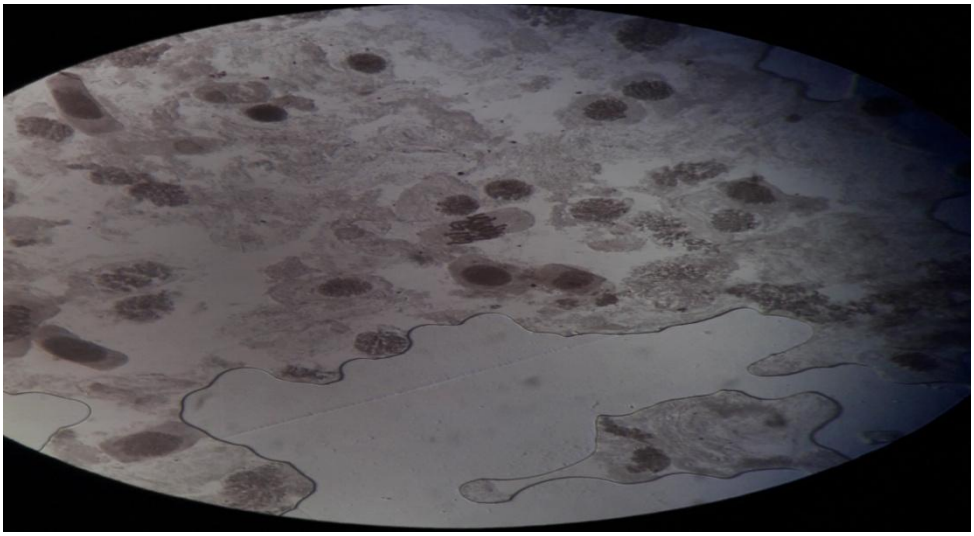

Figure-4c: Chromosomal stickness

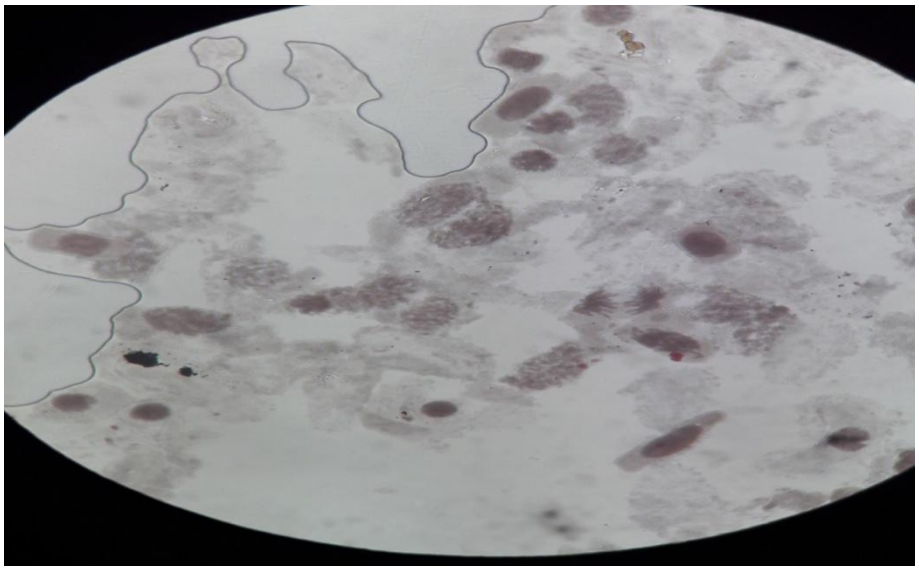

Figure-4d: Chromosomal stickiness

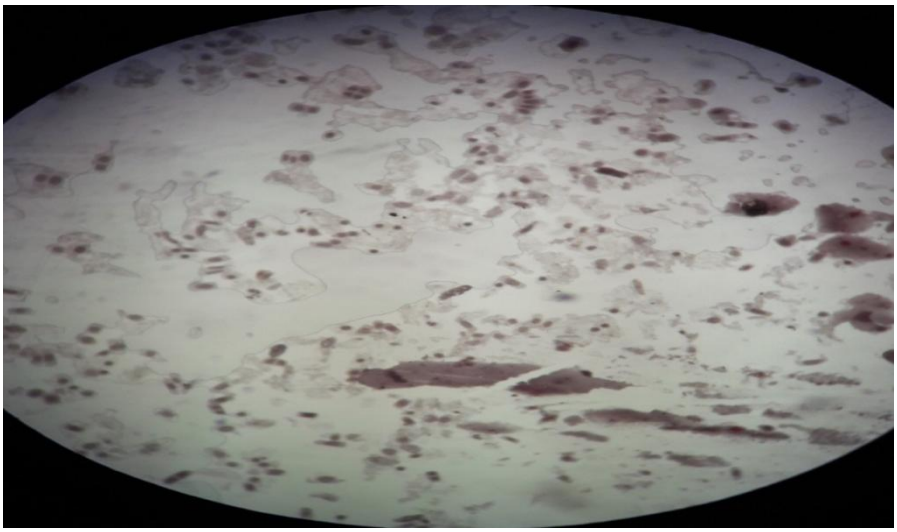

Figure-4e: Polar deviation of Chromosomes

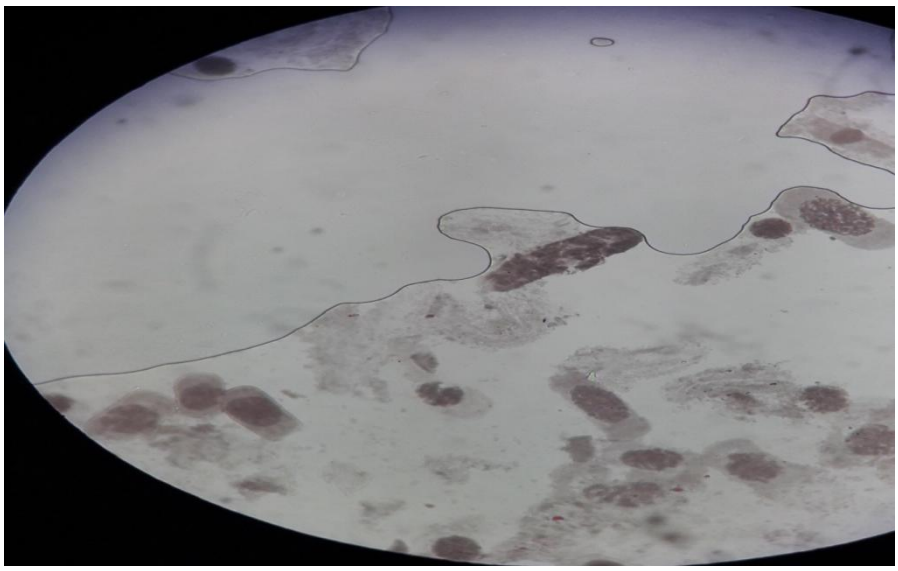

Figure-4f: Polar deviation of Chromosomes and chromatid bridge

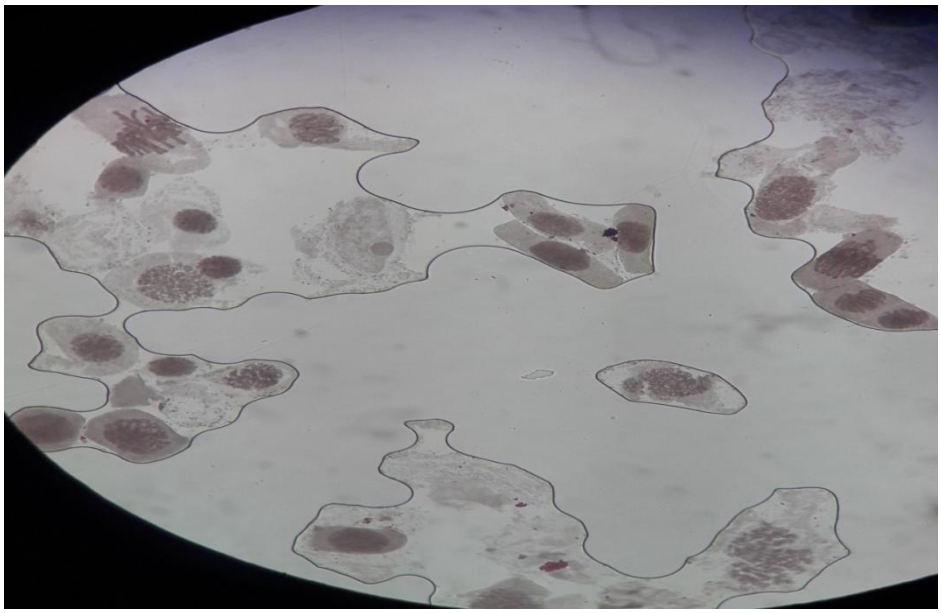

Figure-4g: Polar deviation of chromosomes

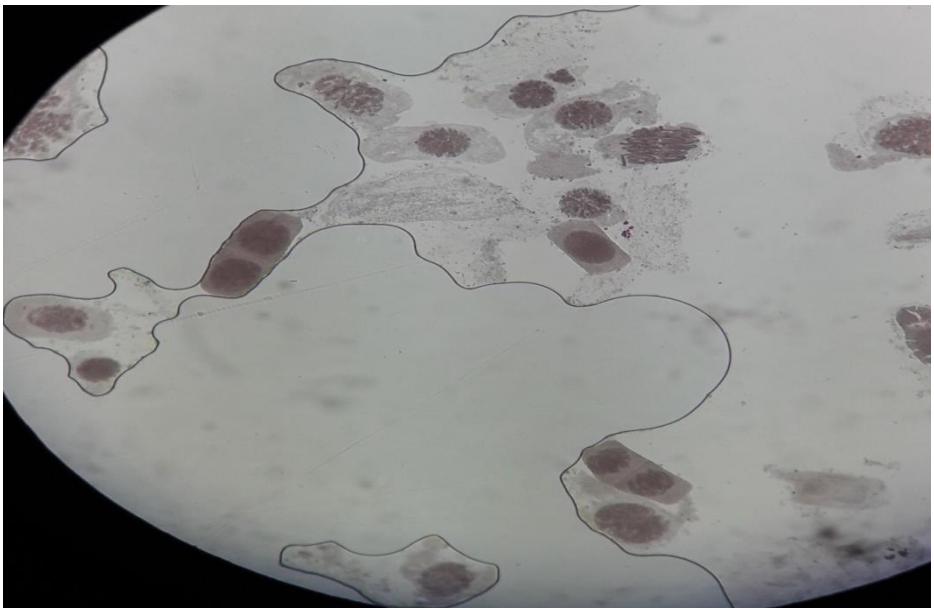

Figure-4h: Polar deviation of chromosomes and chromatid bridge

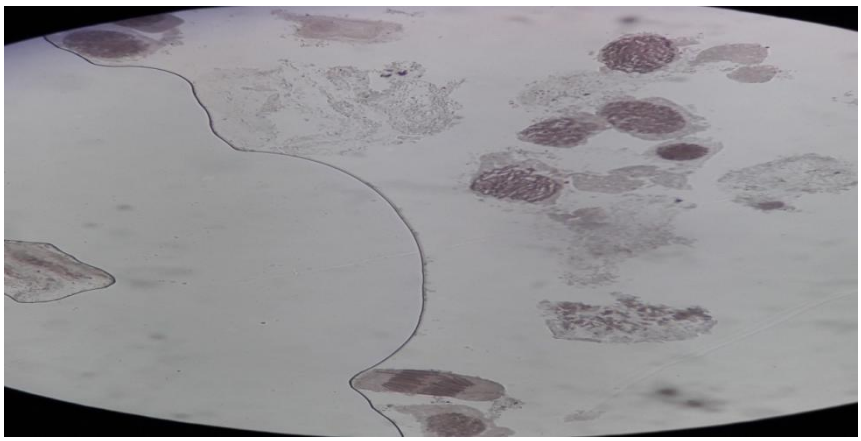

Figure-4i: Membrane damage

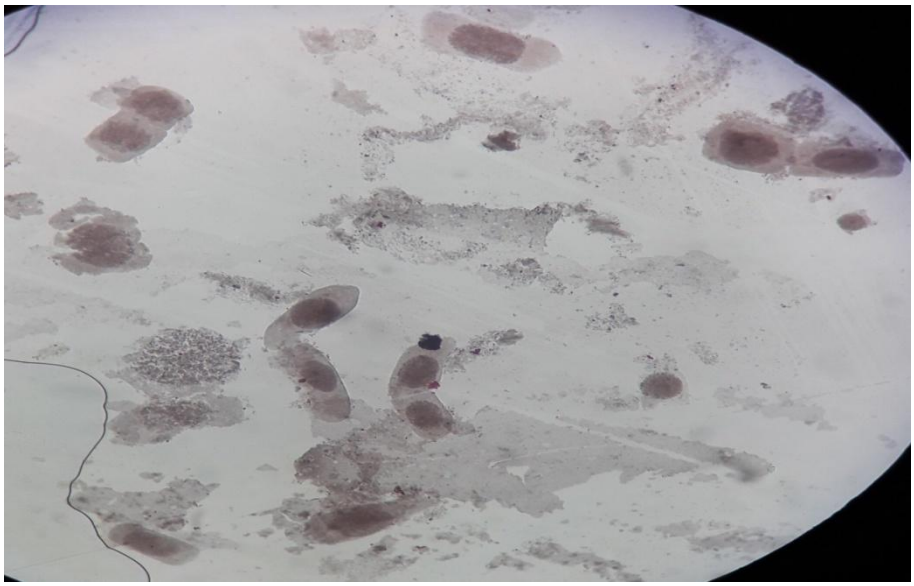

Figure-4j: Membrane damage

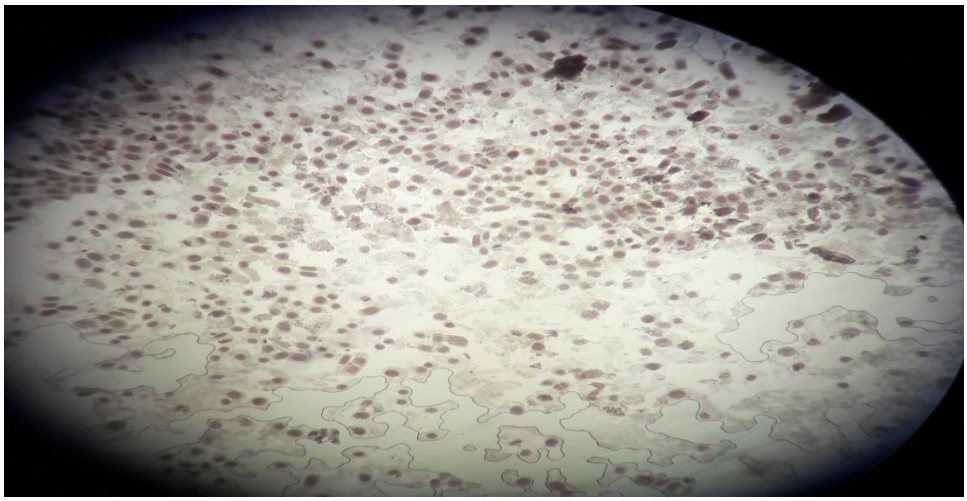

Figure-4k: Micronucei

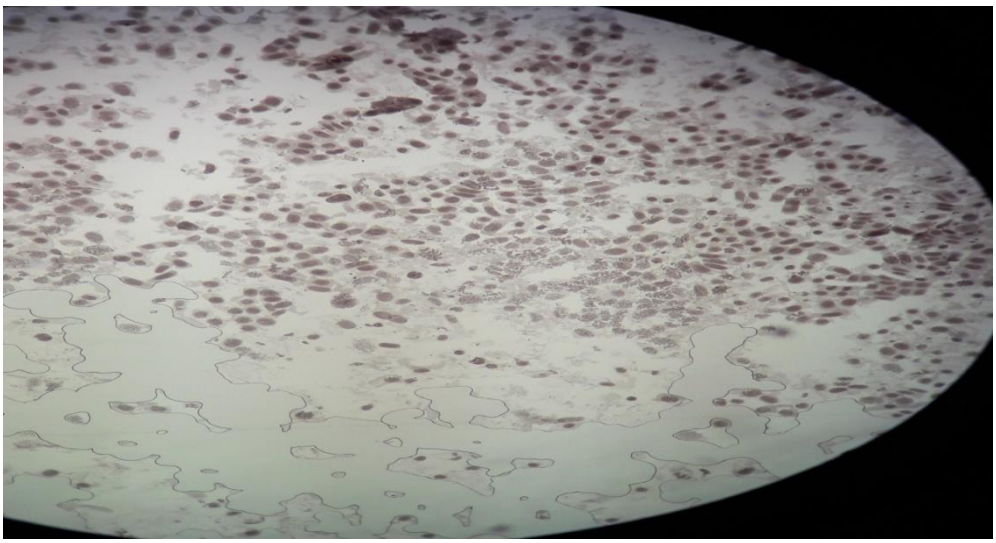

Figure-4l: Binucleate cells

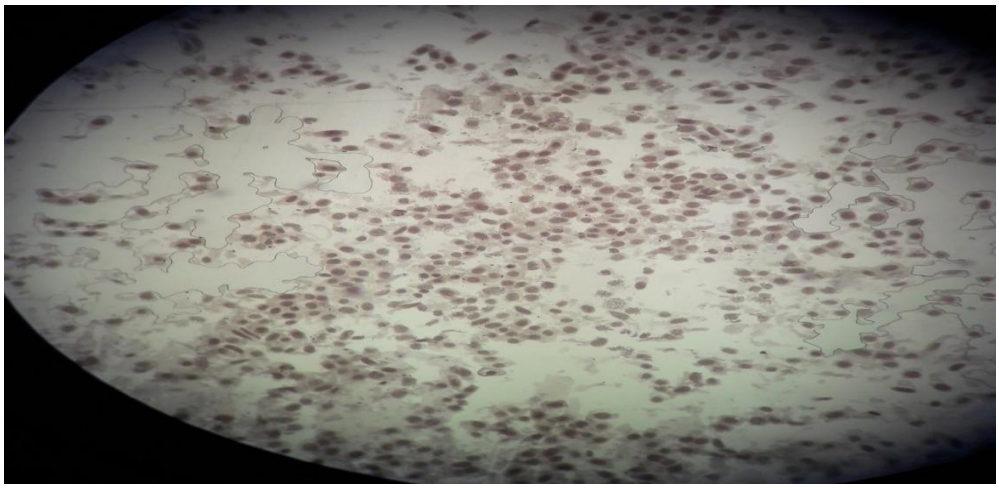

Figure-4m: Binucleate cells

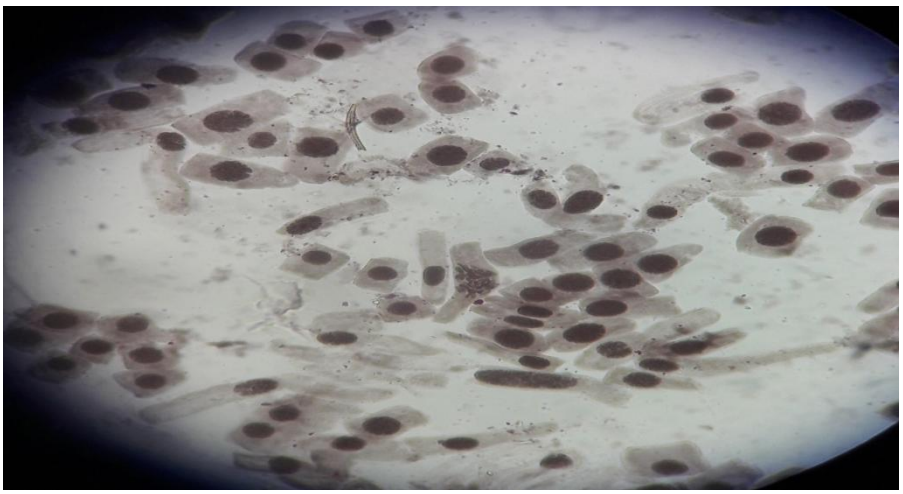

Figure-4n: Aberrant cells

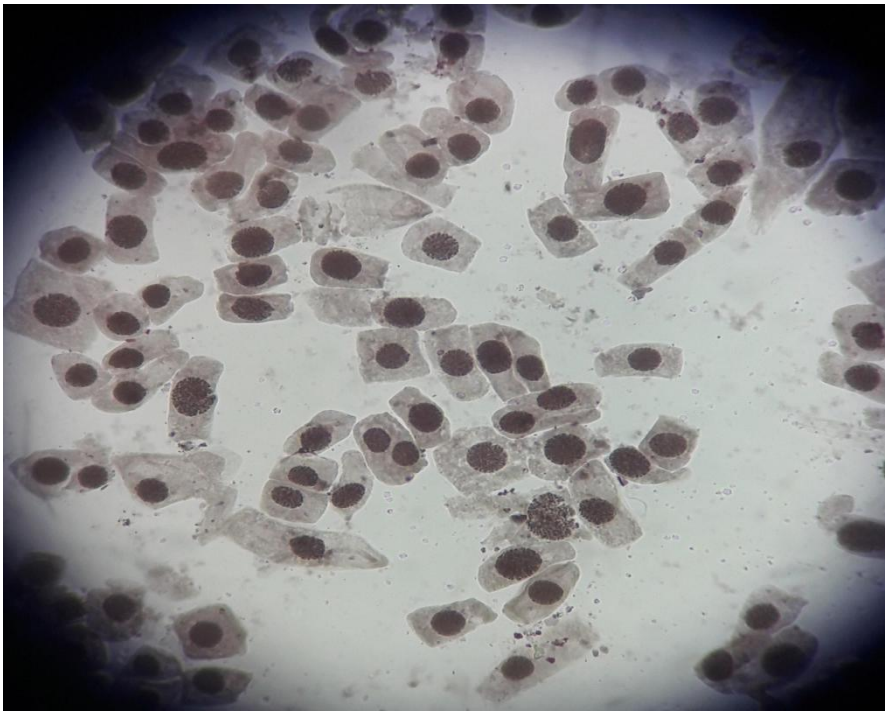

Figure-4o Aberrant cells with damaging nucleus

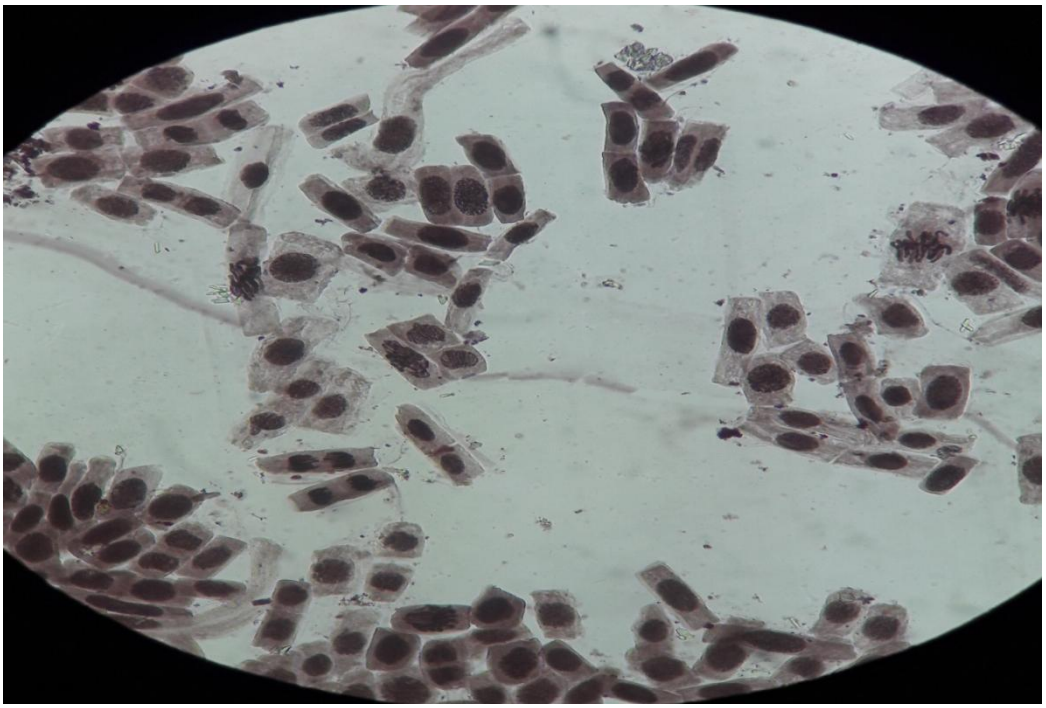

Figure-4p: Aberrant cells in process of dying

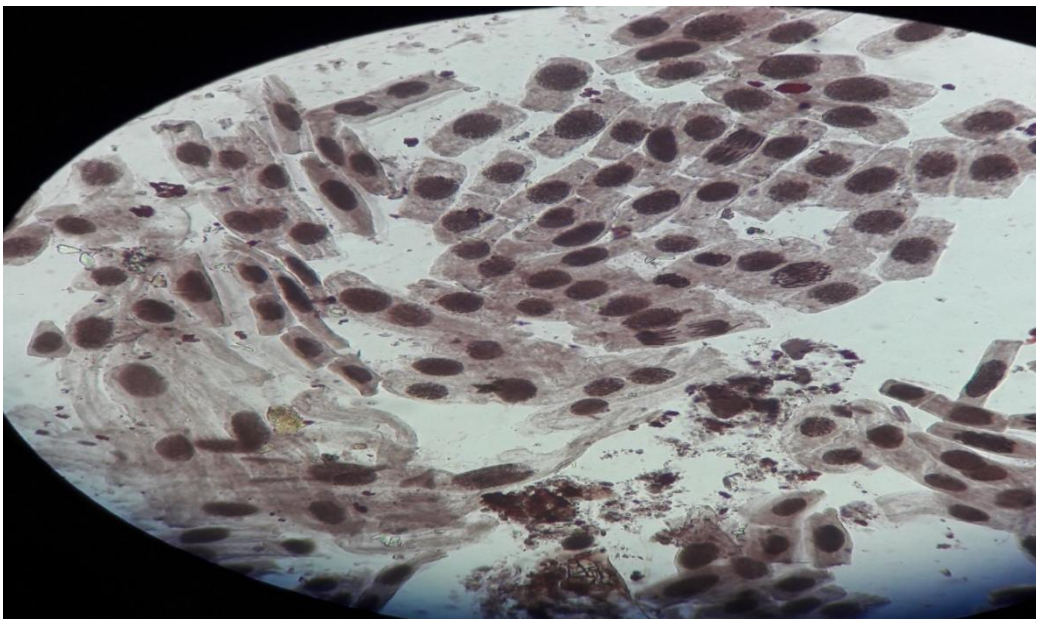

Figure-4q: Aberrant cells in process of dying

Figure-4 a-q: Mitotic and chromosomal aberrations after the treatment of leaf aqueous extracts of medicinal plants in *Allium cepa* root tip meristematic cells as observed under light microscopy.

IV. Discussion

The general cytotoxicity of ten medicinal plant extracts in terms of number of roots and their length on *Allium cepa* growing roots has been presented Table-1. From the results it is evident that the aqueous leaf extracts of all the ten medicinal plants selected for present study caused a significant inhibition in the number and growth rate of *Allium cepa* roots in comparison to negative and positive control. The leaf extract of all the ten plants caused a reduction in the number of roots to about 35.5 to 36.7 at 2.5 mg/ml concentration. The number of roots declined on increasing the concentration of leaf extract. At 10.0 mg/ml concentration the extracts of all the ten plants caused reduction in the number of roots to 20.4 to 24.5. The average growth in length of roots also greatly inhibited on increasing the concentration of leaf extract. *Azadirachta indica* and *Terminalia bellerica* caused maximum inhibition in root length in comparison to negative and positive control.

At 10.0 mg/ml concentration of leaf extracts these two plants caused inhibition in length to about 1.65 to 1.76 (Table-1; Figure-3a-d).

The aqueous leaf extract of all the ten medicinal plants caused strong inhibition of dividing cells and mitotic index (MI) values (Table-2). The total meristematic cells in one microscopic field in negative and positive control were calculated to be 25000. The number of dividing cells in negative and positive control experiments was 1790 and 585 respectively. The mitotic index (MI) in negative and positive control was 7.01% and 2.29% respectively. The aqueous extract of all the ten medicinal plants provoked strong inhibition of dividing cells and MI, where a statistically significant difference in relation to the control and the decrease in the mitotic index was positively correlated with increasing concentration of the leaf extracts. At 2.5 mg/ml concentration the extract caused inhibition in MI value to 3.60% to 3.82% but at concentration of 10 mg/ml of extract there was more or less complete inhibition of MI value (Table-2).

Chromosomal and mitotic aberrations were observed in the root meristematic cells of *Allium cepa* after treatment with aqueous leaf extracts of medicinal plants (Table-3: Figure-4a-q). In negative and positive control experiments no any breaks in chromosomes were observed. The stickness of chromosomes was 0.70% and 32.25% respectively in negative and positive control experiments. The chromosomal fragments detected in different treatments were of chromosome type (Figure4a and b). The chromosomal breaks showed the clastogenic effect of aqueous leaf extracts of all the ten medicinal plants. The occurrence of chromosome fragments showed statistically significant differences at various concentrations of aqueous leaf extracts.

In the present investigation the stickiness of metaphase chromosomes and polar deviations i.e. movement of chromosomes in wrong direction were also observed (Figures-4c, d, e, f, g and h). It was observed that the degree of abnormalities increased with increased concentration of aqueous leaf extracts of all the ten medicinal plants. In this cytotoxicity test on *Allium* a strong toxic effect of aqueous leaf extract was observed. This can be supported by greater degree of stickiness of metaphase chromosome, polar deviations and increased frequency of aberrant cells of leading to cellular death i.e. decreased mitotic index (MI). A statistically significant increase in total aberrant cells ($P < .05$) was observed in comparison to the negative control (Table-3). However, the highest value of aberrant cells was observed in the positive control (41.75 ±8.95%). Statistical analysis showed that the cytotoxicity and genotoxic activities of the aqueous leaf extracts of all the ten medicinal plants induced micronuclei in the root meristematic cells of *A. cepa.* The formation of micronuclei (MNC) in 1000 cells per slide (‰MNC value) was also increased on increasing extract concentrations compared with negative and positive control, which is statistically significant ($P < .05$) (Figure-4k).

In addition, cells with membrane damage (Figure-4i, j, binucleated cells (Figure-4l and m), aberrant cells with damaging nuclei (Figure-4n and o), and the aberrant cells in the process of dying (Figure-4p and q) were also observed in various frequencies. The present findings are in agreement with the work of [27, 28, 29, 30] Tulay Askin Celik and Ozlem Sultan (2009), Essam *et al*., (2016), Khat *et al*., (2018), Khakdan *et al*., (2015) etc. Tulay Askin and Ozlem Sultan (2009) [27] have evaluated the cytotoxicity and genotoxicity of *Inula viscosa* leaf extracts on *Allium cepa*. Essam *et al*., (2016) [28] have studied the genotoxic effect of garlic extract on root tips of *Allium cepa*. Khakdan *et al*., (2015) [30] have evaluated the cytotoxicity and genotoxicity of aqueous extract of *Althea kurdica* on *Allium cepa*. Khat *et al*., (2018) [29] have recently studied the cytotoxic effects of *Chromolaena odorata* on mitosis in onion root tip. All these workers have observed a more or less similar cytotoxic efficacy of aqueous extract on *Allium cepa* root meristem.

In the present investigation the toxic effect of aqueous leaf extracts of ten medicinal plants viz. *Syzium aromaticum, Oxalis corniculata, Solanum nigrum, Azadirachta indica, Bauhinia variegata, Coriander sativum, Anisomeles indica, Terminalia bellerica, Ocimum sanctum* and *Citrus sinensis* was evaluated by analyzing root growth and root morphology. The concentration of extracts caused an inhibition of root growth and there was a statistically significant difference between control groups. Cytototoxicity was studied by observing cytological parameters such as the mitotic index (MI) and number of chromosome abnormalities, including chromosome breaks, stickiness, and polar deviations. The mitotic index (MI) of *A. cepa* meristematic cells treated with the EMS was significantly decreased (2.29% in comparison to negative control). Significant inhibition in the onion roots treated with the aqueous extracts was noticed (Table-2). A positive correlation was found between inhibition of root growth and decrease of MI. The decline of MI below 22% in comparison to negative control can have lethal impact on the organism [31] (Antonsie-Wiez, 1990), while a decrease below 50% usually has sub lethal effects [32] (Panda and Sahu, 1985) and is called cytotoxic limit value [33] (Sharma, 1983). MI measures the proportion of cells in the M-phase of the cell cycle and its inhibition could be interpreted as cellular death or a delay in the cell proliferation kinetics [34] (Rojas *et al*., 1993). Reduction in the mitotic activity could be due to inhibition of DNA synthesis or a blocking in the G2 phase of the cell cycle, preventing the cell from entering mitosis [35] (Sudhakar *et al*., 2001). Mitodepressive effects of some herbal extracts, including the ability to block the synthesis of DNA and nucleoproteins, were reported earlier [36, 37] (Mercykutty and Stephen, 1980; Schulze and Kirschner, 1986). Several other herbal extracts have been reported

to inhibit mitosis [38, 39, 40] (Askin Celik and Aslanturk, 2007; Askin Celik and Aslanturk, 2006; Askinboro and Bakare, 2007).

The decreased MI in *A. cepa* roots treated with aqueous leaf extracts of all the ten medicinal plants might be due to either disturbances in the cell cycle or chromatin dysfunction induced by an external factor. The results suggested that the aqueous leaf extracts of three different concentrations have inhibitory, mito-depressive effects on root growth and cell division of *A. cepa* and it can prevent DNA synthesis and the reduction in number of the dividing cells in roots produced by the cytotoxic effects of phytochemicals found in the aqueous leaf extracts.

The aqueous leaf extracts of all the ten medicinal plants showed the strongest genotoxic effects in the root meristem cells. The appearance of sticky metaphase chromosomes reinforces the hypothesis of the toxic effect of leaf extracts. Metaphases with sticky chromosome, loses their normal appearance, and they are seen with a sticky "surface," causing chromosome agglomeration [41] (Babich *et al*., 1997). Stickiness has been attributed to the effect of pollutants and chemical compounds on the physical-chemical properties of DNA, protein or both, on the formation of complexes with phosphate groups in DNA, on DNA condensation or on formation of inter- and intra chromatid cross links [42, 43, 44, 45, 46] (Shahin and El-Amoodi, 1991; Rencuzogular *et al*., 2001; El-Ghamery *et al*., 2003; Gomurgen, 2005; Torkugly, 2007). Chromosomal aberrations are structural changes in chromosome resulting from a break or exchange of chromosomal material. Most of the chromosomal aberrations observed in cells are lethal, but there are many related aberrations that are viable and that can cause genetic effects, either somatic or inherited [47] (Swierenga *et al*., 1991). The presence of chromosome fragments is an indication of chromosome breaks, and can be a consequence of anaphase/telophase bridges [48, 49] (Sharma and Sen, 2002; Singh, 2003).The induction of chromosome breaks, disturbances on microtubule assembly and cellular death can be related. In the present investigation the frequencies of total chromosome aberrations increased significantly upon exposure to aqueous leaf extracts of medicinal plants which indicate clastogenic activity (Table-3). These results are in conformity with the results of many research groups that examined the effects of different medicinal herbs [50, 51, 52] (Askin Celik and Aslanturu, 2007; Soliman, 2001; Bidau *et al*., 2004).

The aqueous leaf extracts of all plants significantly induced the formation of MNC in *A. cepa* root cells at 2.5–10mg/ml concentrations. Frequencies of MNC increased in 2.5mg/ml and 5 mg/ml of leaf extract. However, MNC frequency decreased in *A. cepa* roots treatment at the highest leaf extract concentration (10mg/ml), due to high cytotoxicity. The frequency of cells with micronuclei is a good indicator of the cytogenetic effects of tested chemicals. Micronuclei often results from the acentric fragments or lagging chromosomes that fail to incorporate into the daughter nuclei during telophase of the mitotic cells and can cause cellular death due to the deletion of primary genes [53, 54] (Albertin *et al*., 2002; Krishna and Hayashi, 2000). Present studies revealed the MNC-induced effect of various plant extracts. Soliman (2001) [55] also reported MNC formation by *Azadirachta indica* A. Juss. aqueous extract treatment on *A. cepa* root tip meristematic cells. Akinboro and Bakare (2007) [56] reported MNC formation by treatment of some *Psychotria* species extracts on *A. cepa* root tip meristematic cells. In the present investigation, membrane damage was observed in groups treated with 5 mg/ml and 10 mg/ml aqueous leaf extracts. In the present investigation, binucleated cells have been observed in 5mg/ml extract treatment group. The occurrence of binucleated cells might be due to inhibition of cytokinesis. Similar inhibition of cytokinesis was also reported by [57, 58, 59, 60] Kaushik (1996), Borah and Talukdar (2002), and Gomurgen *et al*., (2005, Askincelik and Aslanturk, 2009).

V. Conclusions

In the present investigation it can be concluded that the aqueous extracts of medicinal plants when applied in high doses cause cyto-genotoxicity activities. The results suggest that, although these plants have beneficial effects as a medicinal herb, it can cause serious problems and damage on cells when used improperly.

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