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ANTIMITOTIC AND GENOTOXICITY EFFECTS OF WILD LIBYAN ARTICHOKE *Cynara cornigera* LEAVES AQUEOUS EXTRACT

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ABSTRACT

Allium cepa root tip cells were used to find out the effect of Cynara cornigera extract on chromosomal structure and behavior during cell division. To measure the mitotic index and chromosome aberration in treated cells. The root meristem cells were treated with different concentrations of Cynara cornigera leaves aqueous extract (0.1, 0.01 and 0.001 mg/ml) for 4, 6 and 12 hours, respectively. The results indicate that aqueous extracts of C. cornigera plant have the ability to decrease the (MI) values with increasing the concentration at (P<0.005). The treatments have caused different kinds of mitotic abnormalities and chromosomal aberrations, such as: Change percentage of mitotic phases, C-metaphase, Stickiness, Disturbed nuclear membrane, Highly condensation of prophase and Accumulation of cell in interphase.

KEY WORDS: *Bioassay: Cynara cornigera: Leaf extract: Artichoke; Chromosomal aberrations.*

INTRODUCTION

According to the World Health Organization (WHO), about 80% of developing countries use traditional folk medicines, 85% of which are plant extracts. Furthermore, the international center of commerce reported that medicinal plants are still vitally important in the preparation of pharmaceutical products (Fransworth, 1994; Nune and Caravalho, 2003).

Artichoke has been known since the 4th century B.C. as a food and remedy. This plant was appreciated by the ancient Egyptians, Greeks, and Romans, who used it both as a food and as a medicine (for their beneficial effects against hepatobiliary diseases and as a digestive aid) (Marzi *et al.*, 1975; Sonnante *et al.*, 2002).

In Libya, 4 species of Artichoke are reported and they include *Cynara cornigera, Cynara cadunculus*, *Cynara scolymus* and the endemic *Cynara cyrenaica* (Jafri and El-gadi, 1983). *Cynara cornigera* is dwarf plant, 10-20(30) cm and it is flowering in Spring (Plate 1). This plant is distributed in the eastern part of Libya and using widely in folk medicine. Shortage and no enough information about its effect on human use was led to shade some light on this plant. There for, this study was aimed and designed to evaluate the antimitotic and antimutagenic activities of aqueous extract of the leaves of *C. cornigera* on *A. cepa* root tip cells to find out the effect of *C. cornigera* on chromosomal structure and behavior during cell division by studying the mitotic index (MI) in the treated cells.

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Plate (1) showed *C. cornigera* shrub plant in its wild habitat. Note the leaves and its flower in the middle with horns.

MATERIALS AND METHODS

Plant material: *C. cornigera* plants were collected from the borders of Benghazi city and identified according to the mentioned references, beside consultation of the Faculty of Science, Benghazi University, Libya. leaves were washed with distilled water, dried with filter paper in dark place. they were grind by electric blender and were stored as powder in dark flask until used. Commercial variety bulbs of the common onion (*Allium cepa)* were selected in shape and purchased from local market for plant bioassay.

Aqueous extract: 5g of *C. cornigera* powder was added to 100 ml boiled distilled water after cooling to 60 C°. the content of the flask was shaken and left at room temperature (Tawab *et al*,2004). A buchner funnel lined with whatman No.1 filter paper was employed to filtrate the content of the flask. The filtrate was used as stock solution and three different concentration (0.1, 0.01 and 0.001 mg/ml) were prepared by serial dilution method (Jain and Sethi, 1991; Eltorki, 2006; Iranbakhsh *et al*., 2010).

Procedure of plant chromosomes technique: The preparation of plant chromosome achieved using standard methods described by (Grant, 1982), with minor modification.

Onion bulb germination and treatment: Onion bulbs were grown in distilled water at room temperature (25±2 C°) in the darkness. They were placed in small (50 ml) glass cups containing distilled water for approximately 3 to 4 days, when the primary root tips were 0.5-1.5 cm long, the bulbs were transferred to clean and dried containers included the treatments with a series of concentration. In this experiment two groups were used. group 1 included negative control (distilled water) and positive control treated only with *colchicines* solution 0.05%. In treated samples 5 ml of *colchicines* solution 0.05mg/ml were added to each treatment for 3 hours prior to harvesting except the negative control. The group 2 treatment with series of concentration stock solution, 0.1, 0.01and 0.001 for 4, 6 and 12 h. The controls were treated with distilled water only. Ten root tips were cut and examined for each treatment, the experiment repeated for five times.

Fixation and slide preparation: For cytological preparation, the root tips were fixed in 95% ethanol /glacial acetic acid 3:1 (v/v) from 1 to 24 hrs. at 5 C°. The technique which use is described by Fiskesjö (1994). Slides were prepared using the aceto-orcein squash technique, by hydrolyzing the root tips in 1N HCL at 60 C° for 12 min. and stained with aceto-orcein stain for 45min. The meristematic region of the root was removed and washed with 45% glacial acetic acid on glass slide and squashed with flattened glass rod in Glycerol and mounted with cover slip and temporary sealed with clear finger nail polish. Slides from each treatment examined by light microscope under 40x and 100x to find out the mitotic aberrations. The mitotic index MI was calculated as the percentage of dividing cells to the total number of cells examined. Abnormality cells was calculated as the percentage of abnormal cells to the total number of cells examined.

Data analysis: The data of mitotic index MI and C-metaphase of plant chromosomes technique were analyzed by one way analysis of variance (ANOVA), Multiple comparisons were performed by least significance (LSD) using statistical package for social science (SPSS) with significance level less than 0.05 ($P<0.05$).

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RESULTS

Antioxidant activity of *C. cornigera* leaves aqueous extracts

The effects of *C. cornigera* aqueous extract on mitotic index (MI) and c-metaphase of *A. cepa* root tip cells at different exposure time intervals are illustrated in table (1). The obtained data showed significant differences between groups and within group ($p<0.005$). The results of short hours treatments (4 hours) showed significant differences, decrease in MI by decreasing the plant extract concentration compared to (negative and positive) control. Lowest mitotic index values were in samples treated with 0. 001 plant extract, its value was 7.49% compared to the negative control which was 45.60%, the low concentration of 0.001 had more inhibition effect on MI than 0.1 plant extract concentration but close to 0.01 concentration action. The lowest number of c-metaphase cells (2.00) obtained in samples treated with 0.01 plant extract for four hours (4 hours) compared to the positive control which was (26.33) cells in c-metaphase.

Table(1): Mitotic index and C- Metaphase in *Allium cepa* L. root tips treated with *C. cornigera* aqueous leaves extract and 0.05% *colchicines*.

Treatment Mg/ml	4 hours				6 hours				12 hours			
	T.C.	D.C.	M.I.% ± S.D.	C-M ± S.D.	T.C.	D.C.	M.I.% ± S.D.	C-M% ± S.D	T.C.	D.C.	M.I.%± S.D.	C-M± S.D.
Neg. C	1603	731	45.60 ± 13.90	0.00 ± 0.00	1483	493	33.24 ± 8.17	0.00 ± 0.00	1389	378	27.20 ± 14.59	0.00 ± 0.0
Pos. C	1680	525	31.25 ± 11.61	26.33 ± 10.50	1272	356	27.98 ± 2.16	35.33 ± 7.234	1124	169	15.03 ± 5.511	41.00 ± 25.06
0.1 + 0.05 Col.	1317	244	18.53 ± 3.589	5.67 ± 3.51	1464	183	12.5 ± 7.08	1.33 ± 0.577	1876	256	8.90 ± 1.76	4.33 ± 3.78
0.01 + 0.05 Col.	1074	86	8.00 ± 3.83	2.00 ± 1.00	1397	368	26.34 ± 7.50	2.00 ± 1.00	1221	156	12.88 ± 4.36	8.00 ± 5.56
0.001 + 0.05 Col.	1120	85	7.59 ± 4.15	3.33 ± 3.05	1280	382	29.84 ± 7.30	2.67 ± 1.528	1144	160	13.98 ± 5.129	8.33 ± 5.85

T.C. = Total cells **D.C. =** Dividing cells **M.I. =** Mitotic Index **C-M=** c-Metaphase **Neg. C=** Negative Control **Pos. C. =**Positive Control **S.D. =** Standard Deviation **Col. =** *Colchicines*

The data revealed that the decrease in c-metaphase cells co-present with the decrease in mitotic index (MI) of treated cells with plant extract and *colchicines*. These decreases in MI are concentration dependent, while the decrease in c-metaphase cells was concentration independent.

The statistical analysis revealed no significant differences between groups and within groups total cells (TC) at time intervals. But there were significant differences between groups and within groups of dividing cells (DC) at 4 hours treatment with different plant extract concentrations (P= 0.019). There were highly significant inhibitory effects on dividing cells in samples treated with different plant extract concentrations compared to positive and negative control between groups and within groups (P= 0.003) by ANOVA analysis at time of 6 hours.

Table (1) showed a decline in C-metaphase cells at 0.1mg/ml concentration which was 1.33cells in cells treated to 6 hours compared to positive control which was 35.33 cells. By increasing the time to 12 hours, there were significant differences (P= 0.012) between groups within groups on c-metaphase at different concentration ($p<0.05$), but there were not significant differences between groups within groups on dividing cells (P=0.193). ANOVA test indicated significant effect ($p<0.05$) of plant extract on number of c-metaphase in treated sample with all concentrations compared to (positive and negative) control at all-time treatments, also it indicated a significant effect ($p<0.05$) of plant extract on M.I.% in treated samples compared to (positive and negative) control at (4 and 6) hours of time treatment.

Genotoxicity of *Cynara cornigera* leaves aqueous extract

The treatment of *A. cepa* root tip cells with different concentration of *C. cornigera* crude water extract (0.1, 0.01 and 0.001 mg/ml), for different exposure times (4, 6 and 12), the resulted data were presented in table (2).

No abnormal cells were observed at negative control treatment samples at all applied interval times. While at high concentration (positive control)

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of *C. cornigera* leaves aqueous extract, the percentage of abnormal cells were increased (35.735, 51.034 and 44.123 %) by increasing time (4, 6 and 12 hrs), respectively. The percentage of physiological abnormalities at low concentration of 0.001mg/ml were increased from 27.66% at 4hrs treatment to 29.06% at 6hrs and 29.86% at 12 hrs. However, at the time 4hrs of treatment there were increases in abnormality at high concentration of *C. cornigera* extract which were 35.735%, 40.264%, 33.091% and 27.666% at concentration of stock solution of 0.1, 0.01 and 0.001 mg/ml, respectively. Increase of the treatment time to 6, 12 hrs. with high concentration (stock solution) induce an increase in percentage of abnormalities (51.034 and 44.123 %, respectively) compared to 4hrs of time treatment which was (35.735%). The most common type of physiological abnormalities which appeared in all the concentrations and periods of treatment are given in plate (2) and (3) and chromosomal stickiness at metaphase is more observed in all treatments.

Table (2): The effect of *Cynara cornigera* leaves aqueous extract on Mitotic Index % and of Normal cells% and Abnormal cells% of *A. cepa* meristematic cells (Group 2).

Treatment Mg/ml	4 hours			6 hours			12 hours		
	M.I.% ± S.D.	N.C . ± S.D.	Ab. C. ± S.D.	M.I.% ± S.D.	N.C.% ± S.D.	Ab. C. % ± S.D.	M.I.% ± S.D.	N.C. % ± S.D.	Ab. C. ± S.D.
Neg. C.	8.666 ± 0.733	100.0 ± 0.00	000.0 ± 0.00	8.929± 0.559	100.00± 0.00	000.00 ± 00.00	9.535± 1.202	100.00 ± 0.00	00.00 ± 00.00
Stock solution (Pos. C.)	2.244 ± 0.072	64.265 ± 2.04	35.735± 2.042	2.378± 0.194	48.966± 0.32	51.034 ± 0.56	0.323± 0.129	55.877 ± 0.466	44.123± 0.466
0.1mg/ml	3.748 ± 0.28	59.736 ± 1.25	40.264± 1.092	3.230± 0.15	72.39 ± 0.35	27.610 ± 0.615	3.544± 0.374	73.317 ± 0.552	26.683± 0.552
0.01mg/ml	4.792 ± 0.198	66.909 ± 3.89	33.091± 1.358	4.419± 0.322	69.455± 0.355	30.546 ± 0.616	3.983± 0.574	74.183 ± 1.902	25.817± 1.90
0.001mg/ml	5.814 ± 0.826	72.334 ± 0.694	27.666± 0.629	5.601± 0.176	70.936± 0.102	29.063 ± 0.177	5.122± 0.504	70.134 ± 0.178	29.866± 0.178

M.I. =Mitotic Index. **Ab. C. %=**Percentage of abnormal cells. **N.C. =**Percentage of normal cells.
Neg. C. =Negative Control. **Pos. C. =**Positive Control. **S.D. =**Standard Deviation.

Disturbed nuclear shape (stock solution at 12hr).

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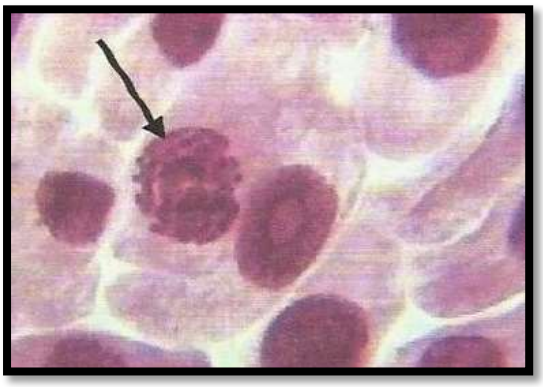
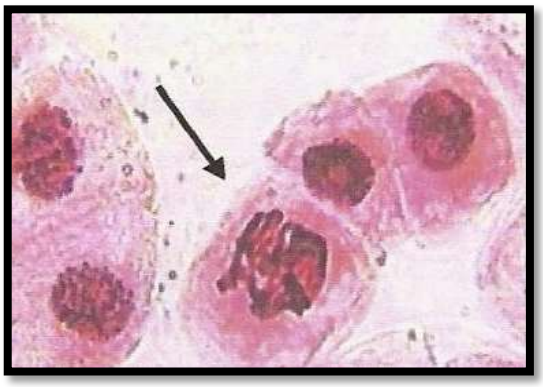
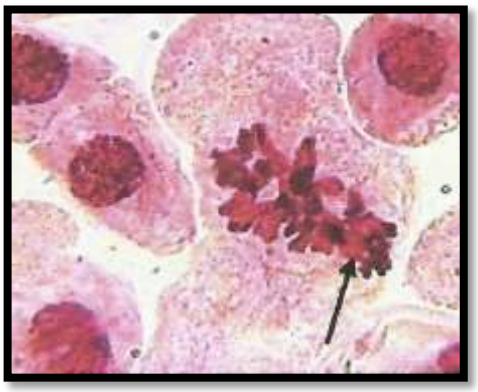


Highly condensation prophase
(stock solution at 4hr).

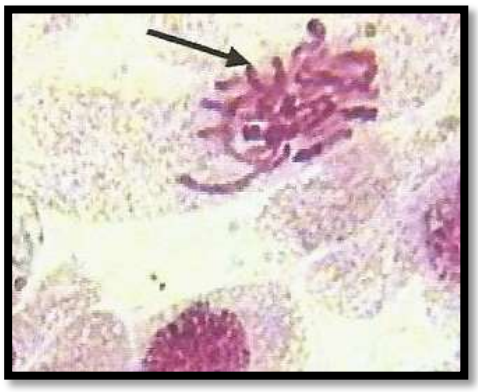


Early condensation propha
(0.1mg/ml at12hr).

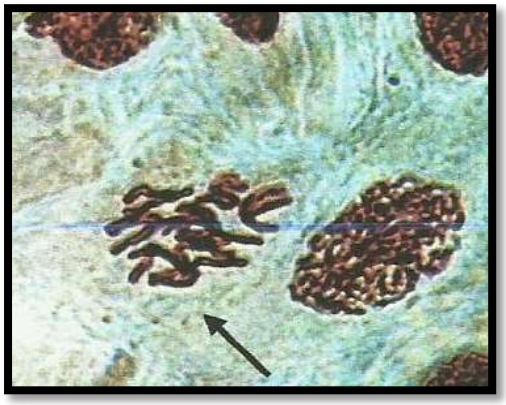
Plate (2) Physiological abnormal mitotic cells of *A. cepa* root tip cells after treatment with different concentrations of *C. cornigera* leaves aqueous extract for different periods.



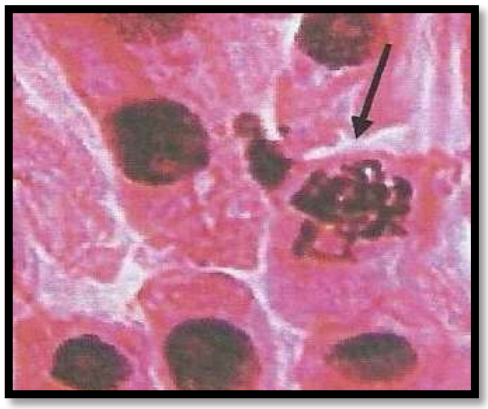
Stick metaphase(0.01mg/ml at 6h).



C- metaphase (positive control at 4h).



C-metaphase (0.1mg/ml at 6h)



Disturbed metaphase (positive control at 12h)

Plate (3) Physiological abnormal mitotic cells of *Allium cepa* tip cells after treatment with different concentrations of *C.cornigera* leaves aqueous extract for different periods.

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DISCUSSION

A. cepa root tip meristems have been widely used for evaluation of cytotoxic and antimitotic activity of various compounds (Fiskesjo, 1988; Soliman and Ghoneam, 2004 and Sehgel *et al.* , 2006). Such mitotic inhibition could result of the inhibition of DNA synthesis which is considered as one of major perquisites for a cell to divide (El Garabulli and Mohamed, 2007). However the reduction of mitotic activity after treatment could be associated with a reduction in the amount of both DNA and RNA (Badr, 1986; Soliman and Ghonium, 2004). Many other investigators attributed the depression in mitotic index values to the inhibition of protein synthesis, or due to inhibition of certain types of nuclear proteins essential in mitotic cycle (Haliem, 1990; Turkoglu, 2008; Ghareeb *et al.*, 1997 and EL-Nahas, 2000). Similar results were obtained by (Medeiros and Takahashi 1987), who reported a decrease in mitotic index in onion root tip cells at all tested concentrations.

The mitotic index values were decreased with increasing of *C. cornigera* concentration and exposure time, especially at high concentration (stock solution) compare to control. This result is in agreement with action of other plant extract proved to be mitodepressive such as: the effects of castor (*Ricinus communis*) seed extract on *Allium cepa* root tip cells, effects of water extract of *Rosmarinus officinalis on Alium cepa* root tips, the mutagenic effects of water extract of ten *Ipomoea* species on root tip cells of onion, genotoxic effect of seed decoction of *Cassia tora* L. (*languminaosae*) in *A. cepa* root tip cells (Borah and Talukder, 2002; Tawab *et al.* , 2004; Mondel *et al.* , 2006, and Solanke *et al.* , 2008), respectively.

The percentage of chromosomal abnormalities decreased at lower extract concentration but it was found to be increased with high concentration. Such results agree with those obtained by Kaushik *et al.*, (1993), who tested water extract of *Datura stramonium* on *A. cepa* root tip cells. They found that the mitodepressive effect of plant extract were increased with increase the concentration and exposure time.

The present experiment also showed that different concentrations of *C. cornigera* extract induced various forms of chromosomal aberration and the aberrant rate goes up with the concentrations. The treatment of *A. cepa* with different concentration of *C. cornigera* water extract induced only physiological types of chromosomal abnormalities which represented by chromosomal stickiness at metaphase and early prophase condensation. No damage of genetic material was observed such as fragment, bridge and micronucleus, which mean the third way suggested by Xiao-wei, 2004, is not the mechanism of *C. cornigera* action of cell dress inhibition.

The percentage of physiological aberration was increased by increased of *C. cornigera* extract concentration and exposure time, these observations agreed with other reports (Ragunathan and Panneerselvam *et al.* , 2007; Solanke *et al.* , 2008; Mahanta *et al.* ,2008). The most common type of physiological abnormality observed in all the concentrations and periods of treatment was, chromosomal stickiness at metaphase, this even may be due to alteration of chromosomal proteins resulting in change in surface nucleoprotein configuration or improper folding in chromosome fiber (Saggoo *et al.*, 1991).

The mitotic index inhibition of root tips of *A. cepa L.* at different concentrations and exposure period times, indicated that *C. cornigera* plant has positive antimitotic effects. The data obtained from this study suggested that using extracts of *C. cornigera* plant is a good and safe method and can be used as antioxidant and anticancer agent. Therefore, more investigation using molecular methods most take in account with more attention.

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