Research article Available online www.ijsrr.org ISSN: 2279–0543

International Journal of Scientific Research and Reviews

Cytotoxic and Genotoxic Assessment of *Citrullus colocynthis*

Qari Sameer H.

Department of Biology, University College, Aljamom, Umm Al-Qura University. P.O. Box 2203, Makkah, Saudi Arabia, sqarinet@gmail.com

ABSTRACT

The present study was planned to clarify the cytotoxic and genotoxic effects of aqueous leave' s extract of *Citrullus colocynthis.* Root tip meristems of *Allium cepa* was treated with different concentrations of aqueous leaves extract of *C. colocynthis*. Samples were taken out at regular intervals for each treatment and subjected to cytogenetic studies including chromosomal aberrations (CA) and micronuclei (MN) assessment, and molecular studies including DNA quantitation, Total soluble protein levels and RAPD-DNA. It was found that the extract has cytotoxic and genotoxic activities at highly concentrations. Mitotic index decreased as concentration or time of exposure increased. Frequency of (CA) and (MN) increased with increase in concentration or exposure time. Most (CA) were disturbance of chromosomes, C-metaphase and binucleate. Pyknotic nuclei cells were highly frequent at high concentration.DNA quantity and total soluble protein levels in seedlings decreased slightly at 23 gm/L of *C. colocynthis*, and inhibited substantially respectively compared with control along with the increase of *C. colocynthis* concentration at 46 and 92 gm/L following 24, 48 and 72h. of treatment. The RAPD results demonstrated a polymorphic numbers of genetic bands, which were the electrophoretic products of PCR for all treatments compared with the control. The obtained results strongly suggest that the leaves extract of *Citrullus colocynthis* is a clastogenic, mutagenic at high dose and anti-carcinogenic agent probably at small dose.

KEYWORDS: *Citrullus Colocynthis,* Aqueous Leave Extract, *Allium cepa, Cytotoxic*, Genotoxic

Corresponding author

Sameer H. Qari

Department of Biology, University College, Aljamom, Umm Al-Qura University.

P.O. Box 2203, Makkah, Saudi Arabia

E-mail: sqarinet@gmail.com. Phone: +966555504086, Fax: +966125399521

INTRODUCTION

Citrullus colocynthis, member of the family Cucurbitaceae, popularly named bitter apple is a well-known medical plant used for many medical purposes. Different parts of the plant including seeds, fruit, root, stem, and leaves, used as either aqueous or oil extracts, dried or fresh. These parts are believed to have antidiabetic[1,2,3] , antihyperlipidemic[4,5], laxative[6,7], anti-inflammatory, analgesic[7] , vermifuge[2], hair-growth-promoting[8] , antibacterial, antifungal[7] , and antioxidant properties[9] .In spite of multiple medical benefits, some of the most frequently reported complications such as colic, diarrhea, hematochezia, nephrosis, vomiting, and liver impairment[10,11] have placed *C. colocynthis* amongst the top 10 toxic plants[11] .

In animals, high doses of the fruits or leaves of *C. colocynthis* have been noted to possess toxicity towards various animals such as sheep[12] and chicks[13], these changes are associated mostly with intestinal damage and lesions associated with bleeding[14] but may be reversible with removal of the food product from the diet[13].

There are various case studies where consumption of high levels of *C. colocynthis* have resulted in intestinal damage such as pseudomembranous colitis[15] acute colitis[16] and anal bleeding (single dose of 1,500-1,600mg of the dried fruits or consumption of a tea made from the plant. These symptoms may be reversible in as little as two weeks of cessation[17].

When using 100mg/L of the dried fruits thrice daily for a period of two months, it was noted that only 13% of participants reported mild diarrhea at the start of the study, which dissipated near the end of the study. There were no other alterations in biochemical or clinical parameters indicating toxicity[6].This lack of alterations in toxicity (assessed by liver enzymes SGOT and SGPT) has been replicated with 100mg of the seed extract thrice daily for six weeks in hyperlipidemia[5] .

These previous studies are generally containing biological activities and systematic toxicity of *C. colocynthis* but there is little information about the cytotoxic, mutagenic and carcinogenic effects. This necessitated a battery of tests to establish its genotoxic effect. Therefore, the purpose of this study is evaluate the genetic effects of the aqueous leaves extract of *Citrullus colocynthis* in root tip meristems of *Allium cepa* by cytogenetic and molecular assays.

MATERIAL AND METHODS

Plant material

C. colocynthis was collected during the last week of May of 2013 from naturally growing plants in their natural habitats located along the belt of *C. colocynthis* in Al-Hajj Street-Makkah; Saudi Arabia, was descripted by[18]. The plant was documented by Herbarium of Umm Al-Qura University, Saudi Arabia.

Preparation of extracts

The leaves washed well with running water to get red of dust and sands, hand-minced into small pieces, mixed with sterilized distilled water and further blended in a blending machine. The mixture left for 24 hours at room temperature with mild hand shaking at regular intervals, then filtered through a membrane filter and the extract (filtrate) either used directly in the experiment or refrigerated for no longer than 3 days for future use. Three concentrations of this extract were prepared (23, 46, 92 gm/L) be tested for genotoxic activities based on the EC50 of *Allium cepa* treated with different concentrations of aqueous leaves extract of *C. colocynthis*.

Chemicals

All chemicals used in the present study obtained from Sigma, except the followings: DNA polymerase (Perkin-Elmer Cetus), dNTPs (Boehringer Mannheim), DNA purification and extraction reagents, and Agarose gel (Qiagen), Oligonucleotides as random primers (Genetic laboratory, National Research Center (NRC), Egypt), DNA Maker for agarose gel electrophoresis (Gibco BRL), loading dye solution (Fermentas, Lithvuania), protein assay reagents, electrophoretic reagents, and protein standards (Bio-Rad).

Treatment

1- **Root growth inhibition test and determination of Effective Concentration for 50% growth inhibition (EC50) using *A. cepa*:** Dose selection experiment was conducted with different concentrations of aqueous extracts of the wild plant *Citrullus colocynthis* leaves (20, 30, 50, 100, 200, 400 and 600 gm/L). The extracts were prepared just before each treatment. For toxicity, study seeds of *A. cepa* obtained from local market were used thoroughly rinse with tap water and after that with distilled water. After cleaning the seeds were placed between two sheets of filter paper and dried at 25°C. Twenty seeds with the same shape and size were placed on filter paper in each of 4 Petri dishes (11 cm in diameter). Five milliliter of each of seven extracts or distilled water, as a control, was applied to the seeds. The dishes were sealed and

incubated at 25±1°C for four days. The length of the roots of germinated seeds was measured in mm. A growth curve was drawn based on the obtained values in a diagram. From the growth curve EC50 value was obtained: The effective concentration causing 50% growth inhibition in relation to control[19]. Three replications were done.

2- Cytogenetic analysis (*Allium cepa* test): We choose to test the cytotoxic and genotoxic effects of three concentrations of aqueous extracts of the wild plant *C. colocynthis*, representing ¼EC50, ½EC50 and EC50 after 24, 48 and 72 h exposure. Thirty *Allium cepa* seeds (2n = 16) purchased from a local market were placed on filter paper in Petri dishes containing 5 mL of distilled water. The dishes were sealed and incubated at 25±1°C for 72 h. twenty germinated seeds with equal length of roots (~ 1 cm) were removed and placed on filter paper in each of another four Petri dishes. Five mL of each concentration of aqueous leaves extract of *Citrullus colocynthis* were added to one dish and incubated at 25±1°C for 24 h. Distilled water was used as control. The same procedure was repeated in the second experiment but the duration of exposure was 48 h. and 72h. respectively.

3- Chromosomal analysis: aberrations and micronuclei in *Allium cepa* root cells were assessed by light microscopy[20]. At the end of the 24, 48 and 72 h exposure the roots were fixed in Carnoy's fixative (95% ethanol: acetic acid glacial, 3:1) for 90 min, hydrolysed in 3N HCl for 8 min and in 45% acetic acid (CH_3COOH) for 30 min at room temperature and stained for 90 min in 4% acetocarmine. After staining, the terminal root tips (1-2 mm) were cut off and squashed in 45% CH_3COOH. The endpoints measured were mitotic index, index of each phase of mitotic division, chromosomal aberrations and cells with micronuclei. Each sample consisted of three root meristems. At least 1000 cells of each root meristem were analyzed. The mitotic index was determined as a ratio between the number of cells in mitosis and the total number of analyzed cells (~ 3000)**.** The index of each phase of mitotic division was calculated as a ratio between the cell number in the respective period and the number of dividing cells. The categories of aberrations scored in mitotic cells included stickiness, disturbance of chromosomes, C- metaphase, binucleate, bridges, and leakage. The percent of micronuclei cells were also detected[21].

4- Total soluble protein level test of seedlings and DNA extraction: The mature seeds of *Allium cepa* were soaked at 4 °C for 2–3 days in the distilled water, and germinated to primary roots of 2-4 mm long in a petri dish containing a filter paper of 11 cm diameter at temperature of 15-24 °C in the dark. Uniformly 25

germinated seeds were selected and transferred to Petri pots containing cultivar sand with 150 ml of distilled water or test extraction (23, 46, 92gm/Lof *C. colocynthis*). Petri pots were incubated in a growth chamber at temperature of 24 ± 1 °C for 24, 48 and 72 h. then, the total soluble protein level of root-tips in plantlets were measured[21,22].

5- DNA extraction, approximately 50 seedlings for each treatment were collected, ground in liquid nitrogen, and total genomic DNA was extracted accordingly to **Scott *et al*[23]** with minor modifications. Purified DNA concentration and integrity of total genomic DNA in each sample were estimated fluorometrically by Biophoto meter (Germany) and by observing EB-stained band with DNA standard of 2 kb.

6- RAPD profile: The conditions of DNA amplification were optimized[24] with some modifications. PCRs were performed in reaction mixtures of 25 µl containing approximately 70 ng of genomic DNA dissolving in sterile distilled water, 1.7 µM primer, 200 µM dNTPs (50 µM of each), 10× reaction buffer (100 mM Tris-HCl, pH 8.3, 15 mM $MgCl_2$, 500 mM KCl, 0.1 mM EDTA, 5 mM DTT, 50% glycerol, 0.1% Triton X 100) and 2.2 U of Taq DNA polymerase. The 6 primers used were of 10 bp in length (Table 1). The RAPD protocol consisted of an initial denaturing step of 5 min at 94 °C, followed by 35 cycles at 94 °C for 30 s (denaturation), 38 °C for 60 s (annealing) and 72 °C for 60 s (extension), with an additional extension period of 10 min at 74 °C. A negative control, without genomic DNA, was run with every set of samples to confirm that no contaminating DNA was present in the reactions. Amplification was carried out in a thermocycler (Perkin-Elmer model 480) with heated lid. Amplification mixtures were stored at 4 °C before use. The reproducibility of the RAPD profiling method in detecting *C. colocynthis* induced DNA changes was also determined using three replicates, and this experiment was also conducted on to confirmed if extra band appeared in majority of *Allium cepa* seedlings exposed to 23, 46 and92gm/L of *C. colocynthis* for 48h[21].

Table 1: Sequences of 6 primers used in this experiment

Primers		**Sequences of primers (5′ → 3′)**
No.	**Name**	
1	OPB-16	TTT GCC CGG A
2	OPA-04	GTC GAA CGA G
3	OPC-20	ACT TCG CCG A
4	OPE-03	CCA GAT GCA C
5	OPA-02	AGC CTT CGC T
6	OPB-03	CAT CCC CCT G

7- Gel electrophoresis: PCR reaction products were mixed with one-sixth volume of gel loading buffer (analytical grade water containing 36% glycerol, 0.05% bromophenol, 30 mM EDTA and 0.05% xylene cyanol), and then separated by electrophoresis in a 1.4% agarose gel, using a Tris-borate-EDTA (TBE) system (0.5 × TBE = 45 mM Tris-base, 45 mM boric acid, and 1 mM EDTA). Agarose gel dimensions were 12 × 6 × 0.5 cm^3. For comparison, DNA molecular size marker (2 kb) was used for each agarose gel. In all PCR-gels, the marker bands visualized were, from top to bottom, 2000, 1000, 750, 500, 250, and 100 bp. Electrophoresis was carried out at temperature of 4 °C at 60 V for 2 h in a 0.5 × TBE buffer and after which the gels were stained with EB solution (0.015%) in distilled water for a period of not less than 25 min. The size of each amplification product was automatically estimated using the gel documentation system (Vendor, Italy). Images were captured using system camera.

8- Statistical Analysis: Data was presented by One-way analysis of variance and Tukey's significant difference test were used for multiple comparisons of data. Linear Regression were calculated for the detection of linear relationship between concentrations of *C. colocynthis* and exposure time for each treatment, using Stat-graphics V 11.0 and Microsoft Excel 2013 for Ms-Windows. Mitotic index (MI)and Mutational frequency (MF) were analyzed by calculation as follow: MI=(total dividing cell [TDC÷] 3000 [total observed cells])×100. Mutational frequency (MF)=((total defected mitotic phases [TDMP]÷3000)×100)÷MI. Inhibitory rate (%) was calculated by the formula $(1 - x/y) \times 100$, where y was the average value detected in the control and x was one in each samples treated. Three replicate were set up for each treatment and the experiments were carried out in duplicate for comparison**.**

Genomic template stability (%) was calculated as $(100\ a/n)$, where a was RAPD polymorphic profiles detected in each samples treated and n the number of total bands in the control. Polymorphism observed in RAPD profiles included disappearance of a normal band and appearance of a new band in comparison to control RAPD profiles[25,26].

RESULTS

Determination of effective concentration for 50% growth inhibition (EC50)

The dose of 20 and 30 gm/L caused only a slight negative effect- inhibition of root length by 5.8 and 6% compared with the control. The growth inhibitory effect increased with elevated concentrations of *C. colocynthis*, the differences with the control were 18.7% (50 mg/L), 56.2% (100 mg/L) and 61.3% (200

mg/L). I observed much stronger inhibition of root growth at the concentrations of 400 mg/L (differences with the control of 79.8%) and 600 mg/L (differences with the control of 95.2%). The effective concentration of the extract of *C. colocynthis* that cause 50% of root length as compared with control (EC50) was **92.0gm/L** approximately.

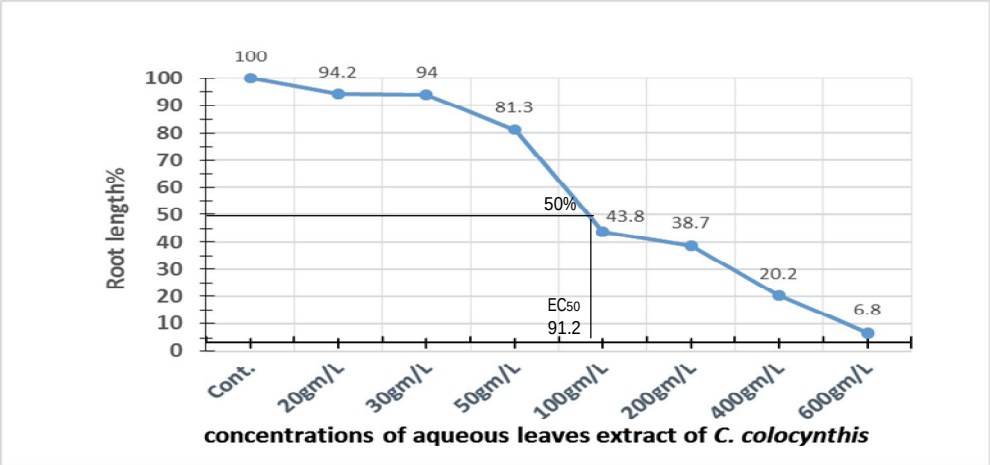

Figure 1: Effective concentration for 50% growth inhibition (EC50) of *C. colocynthis* extract for 96 h on root growth of *A. cepa*.

Cytogenetic analysis (table 2 & figures 2-4)

Chromosome aberrations (CA) were observed in most phases of mitosis. Frequencies of chromosome aberrations induced by treatments. *C. colocynthis* leaves extract showed concentration-dependent increase in the frequency of chromosome aberrations. At high concentration (46 and 92 gm/L), fragments, sticky and disturbance chromosomes were the most common chromosome aberrations observed. Other chromosomal abnormalities observed were c-mitosis, bridge, and binucleate. *C. colocynthis* at 46 and 92 gm/L showed high as much percentage mutational frequency as compared to control. The mutational frequency (MF) values were increased significantly ($p < 0.01$) at 23 gm/L for 72h., and at 46 and 92 gm/L for all durations treatments.

The induction of micronucleus formation was generally observed in 46 and 92 gm/L concentration of C. *colocynthis* and significantly different when compared with the control. Micronucleus formation was markedly higher at 46 gm/L than at the other concentrations (Table 2).

Table (2) also shows the cytological effects of *C. colocynthis* leaves extract on meristem cells of *A. cepa*. Exposure of C. *colocynthis* extract inhibited the mitotic index in a concentration and time-dependent

manner when compared to the mitotic index of 8.7 in the control. The lowest Mitotic Index (MI) value of 3.5 was recorded for 92 gm/L treated with *C. colocynthis* extract for 72h. The mitotic index for *C. colocynthis* extract decreased significantly ($p < 0.01$) at 46 and 92 gm/L compared with control.

These cytogenetic results suggest cytotoxic, genotoxic effects of *C. colocynthis* leaves extract on meristem cells of *Allium cepa*. The microscopic study recorded an extensive cell death (Pyknosis), which is known as a necrosis in animal tissues.

Table 2: Frequency of mitotic phases, Mitotic index and Percentage of clastogenesis abnormalities and mutational frequency in root meristems of *Allium cepa* treated in different duration times with *C. colocynthis* leaves extract (23,46 and 92 gm/L.) for 24,48 and 72 h.

Sample	Time of treatments (h	Interphase (%)	Normal Phases				TDC	MI	TDMP	Clastogenesis								TAC	MF
			Prophase	Metaphase	Anaphase	Telophase				Micronuclei	Brake and Fragments	Bridges	Stickiness	C-Metaphase	Disturbance	Binucleate	Pyknosis Cell		
Control	0	91.3	160	31	20	51	262	8.7	5	0	0	0	4	0	1	0	0	0.17	0.019
2.3%	24	91.7	148	37	23	42	250	8.3*	6	0	0	1	2	0	2	1	0	0.20*	0.024*
	48	91.5	150	80	19	7	256	8.5*	4	0	0	0	3	0	1	0	0	0.13*	0.016
	72	93.9*	131	28	11	12	182	6.1**	17	4	2	0	4	0	5	2	0	0.57**	0.094**
4.6%	24	92.6*	128	39	25	30	222	7.4**	40	9	11	5	6	0	0	1	8	1.30**	0.180**
	48	93.6*	130	28	17	16	191	6.4**	46	17	13	1	2	0	3	0	10	1.50**	0.240**
	72	94.4*	139	15	10	5	169	5.6**	38	14	9	0	1	0	2	0	12	1.20**	0.224**
9.2%	24	95.1**	126	5	7	6	146	4.9**	39	6	7	0	1	0	0	2	23	1.30**	0.270**
	48	95.3**	101	17	8	16	142	4.7**	50	0	0	0	1	6	0	3	41	1.67**	0.350**
	72	96.5**	94	10	0	0	104	3.5**	52	2	0	0	0	0	0		50	1.73**	0.500**

TDC: Total Dividing cells; MI: Mitotic Index; TDMP: Total Defected Mitotic Phases; TAC: Percentage Total of Abnormalities Cells; MF: Mutational frequency $^{*}P < 0.05$. $^{**}P < 0.01$. Total observed cells (3000).

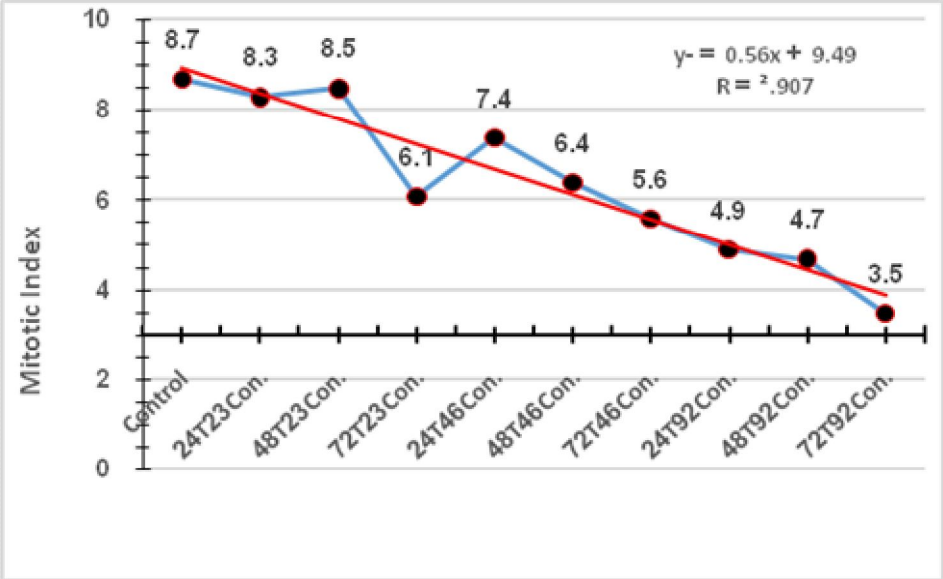

Figure 2: Mitotic index of root tip cells of *Allium cepa* that which treated by leaves aqueous extract of *C. colocynthis* (23,46 and 92 gm/L.) for deferent duration times (24,48 and 72 h).

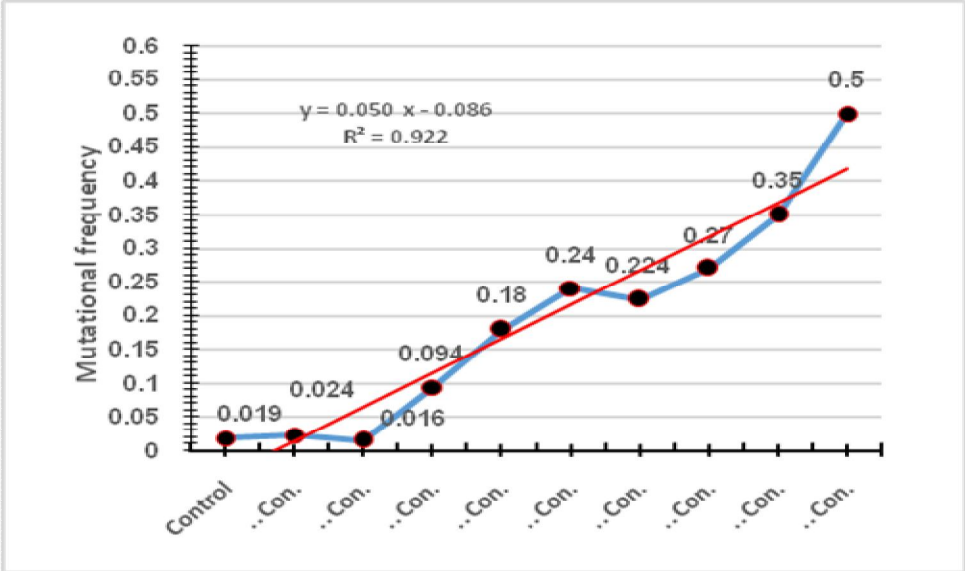

Figure (3): Mutational frequency in root tip cells of *Allium cepa* that which treated by leaves aqueous extract of *C. colocynthis* (23,46 and 92 gm/L.) for deferent duration times (24,48 and 72 h).

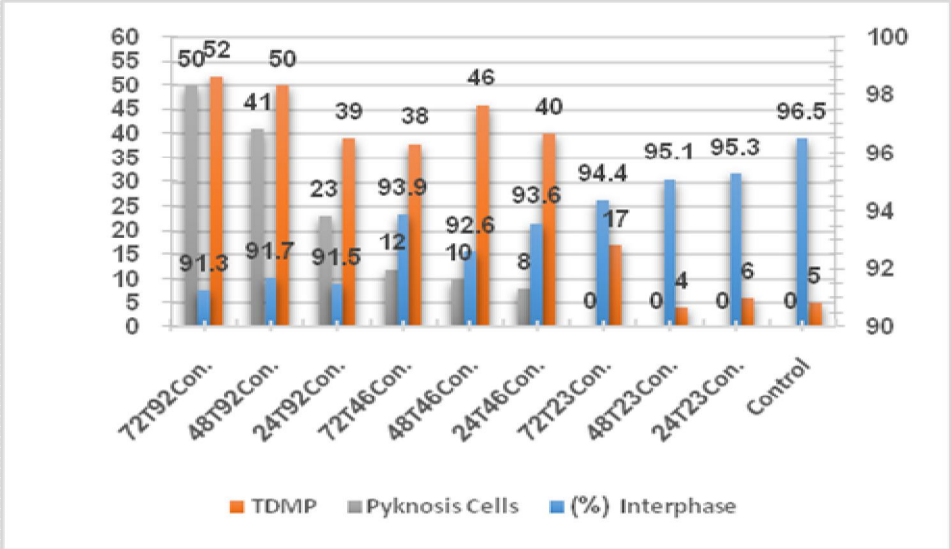

Figure (4): Relationship between concentration of aqueous extract and percentage of mitotic phases of root tip cells of *Allium cepa*.

Effect of C. Colocynthis on DNA quantity and total soluble protein content of root tips in Allium cepa seedlings (table 3& figure 5)

DNA quantity and total soluble protein levels in seedlings decreased slightly ($P > 0.05$) at 23 gm/L of *C. colocynthis*, and inhibited substantially ($P < 0.01$) respectively compared with control along with the increase of *C. colocynthis* concentration (46 and 92 gm/L). following 24, 48 and 72h. of treatment. There was a negative correlation between *C.*

Colocynthis concentration and DNA quantity or total soluble protein content in *Allium cepa* seedlings; with a correlation coefficient of (r^2) 0.922 and 0.820 respectively.

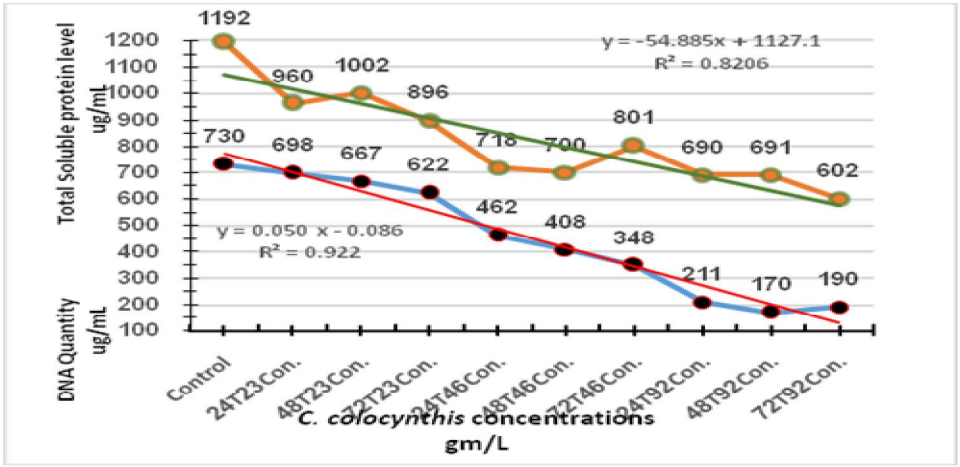

Figure 5: negative correlation between and total soluble protein content in *Allium cepa* seedlings

Table 3: Effect of *C. colocynthis* on DNA quantity and total soluble protein content of root meristems of *Allium cepa* seedlings after exposure to elevated concentration of *C. colocynthis* in deferent duration times.

No.	Treatments		DNA Quantity µg/mL	Total soluble protein of root-tips	
	Sample	Time (h.)		Total Soluble protein level µg/mL	Inhibitory Rate (%)
1	Control	Control	730	1192	0
2	2.3%	24	698*	960*	19.46
3		48	667*	1002*	15.93
4		72	622**	896**	24.83
5	4.6%	24	462**	718**	39.76
6		48	408**	700**	41.27
7		72	348**	801*	32.50
8	9.2%	24	211**	690**	42.11
9		48	170**	691**	42.03
10		72	190**	602**	49.49

*$P < 0.05$,
** $P < 0.01$.

The RAPD - DNA profile:

Only five primers out of the six random primers tested, were gave specific and stable results are presented in Table (4). The RAPD fingerprints showed substantial differences between control and treatment plantlets, with apparent changes in the number and the intensity of amplified DNA bands. The decrease in band intensity was particularly obvious for *Allium cepa* exposed to 46 and 92 gm/L of *C. colocynthis* for primer 1, 2, 3, 4 and 5 are showed in Figure (6). In contrast, an increase in band intensity occurred mainly for all *C. Colocynthis* concentration for all primers but was greater at primer number four as appear in Table (4). The number of disappearing RAPD bands was recorded for all treatments of *C. Colocynthis* concentration, but was greater at 46 gm/L for all primers, and bands of molecular size from approximately 650 to 1050 bp were shown to disappear. Finally, extra bands appeared with primer 1 (five new bands), primer 2 (tow new bands), primer 3 (zero RAPD band), primer 4 (three new bands) and primer 5 (one RAPD band) as showed in Figure (6) and Table (4). Extra bands of molecular size from approximately 250 to 940 bp were indicated to appear Figure (6). Results suggested that new bands in Table (4) were highly appearance at 46 gm/L of *C. colocynthis* for primer 1. Alternatively, that new RAPD bands intensity, stable and specific were changed with the increase of *C. colocynthis* concentration.

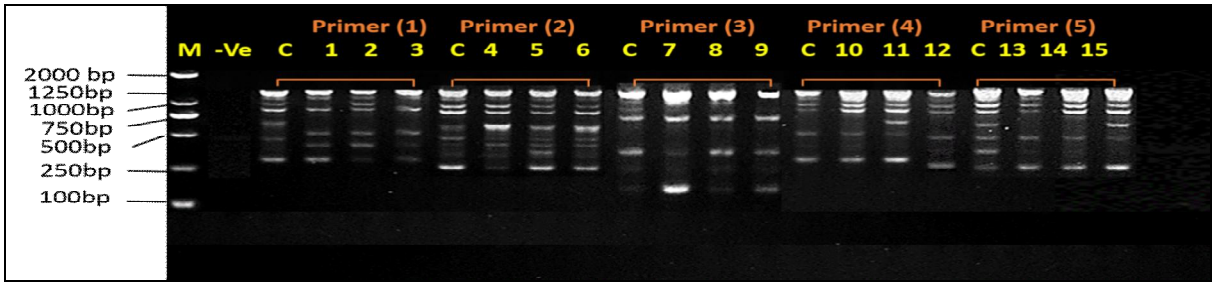

Figure 6: RAPD profiles of genomic DNA from root-tips of *Allium cepa* seedlings exposed to varying *C. colocynthis* concentration (23, 46, 92 gm/L) for 48h. Header photo was indicated by M: DNA marker, -ve: negative gel control, C: control patterns, (1-15): treatments for five primers.

Meanwhile, 5 primers gave a total of 39 bands ranging from 230 (primer 3) to 1260 bp (primer 5) in molecular size in the normal seedlings (as a control). Different polymorphic bands were detected at each concentration of *C. colocynthis* for different primers. Value of polymorphisms was *P* (%) = 25.64%, 33.33% and 23.07% for 23, 46 and 92 mg/L of *C. colocynthis*, respectively. In all cases, polymorphisms were due to the loss and/or gain of amplified bands in the treated samples compared with the control.

Table 4: Changes of total bands in control, and of polymorphic bands and varied bands in *C. colocynthis*-contaminated *Allium cepa* seedlings

Primers		***C. colocynthis* concentration (mg/L)**													
No.	Name	0	23				46				92				
			a	b	c	d	a	b	c	d	a	b	c	d	
1	OPB-16	8	2	3	0	0	3	3	1	1	2	2	1	1	
2	OPA-04	10	0	0	2	2	1	2	3	1	0	0	1	1	
3	OPC-20	7	0	1	1	1	0	0	1	0	0	2	0	1	
4	OPE-03	6	0	1	0	3	1	1	0	4	2	0	0	1	
5	OPA-02	8	1	2	2	1	0	2	0	1	0	1	2	2	
Total bands		39	3	7	5	7	5	8	5	7	4	5	4	6	
a + b			10				13				9				
a + b + c + d			22				25				19				

a: indicates appearance of new bands, b: disappearance of normal bands, c: decrease in band intensities, and d: increase in band intensities. a + b denotes polymorphic bands, and a + b + c + d, varied band.

DISCUSSION

Determination of effective concentration for 50% growth inhibition (EC50):

The results from present study revealed concentration-dependent and statistically significant inhibition of root growth (EC50) of *A. cepa* by *C. colocynthis* at concentrations above 92.0 gm/L when compared with the control.

Cytogenetic analysis (Allium cepa test):

Higher plants such as *A. cepa* are accepted as commendable genetic models to evaluate cytotoxic and genotoxic effects such as chromosome aberrations, micronucleus, mitotic index, and mutational frequency in the mitosis. Results of the present study supported the benefit of *A. cepa* root tips cells for assessment of genotoxic effects of plant extracts[27].Several types of chromosome aberrations were considered in the four phases of mitosis (prophase, metaphase, anaphase and telophase) to evaluate chromosomal abnormalities and mutational frequency. According to[28,29], chromosome aberrations analysis not only allowed estimation of genotoxic effects, but also enabled evaluation of their clastogenic and molecular actions.

Chromosome aberrations presented important information and may be considered an efficient test to investigate the genotoxic potential of the treatments analyzed[30]. The most chromosome aberrations observed were stickiness and disturbance. These aberrations were due to the effect of the extract on the spindle formation and thus resulted in cell division disturbances. Chromosomal fragments indicating the clastogenic effect caused by chromosome breaks, whereas bridges and c-metaphases increase the risk for aneuploidy[31]. Chromosome bridges were observed during anaphase and telophase. The bridges noticed in the cells were probably formed by breakage and fusion of chromatids or subchromatids[32]. A low frequency of c-mitosis that may be attributed to the failure of the spindle apparatus to organize and function in a normal way. Similar observations have been made by other studies[33,34]**.** However, these changes may induce the formation of polyploidy cells when not reversed[35]. Binuclear cells that were induced with significant frequency, they are indicative of the ability of one substance of C. colocynthis extract to interfere with spindle and cell wall formation, a similar cytogenetic results were obtained by[36]**.**

The micronucleus in interphase cells was determined. The percentage of micronuclei cells was obviously higher than control ($p < 0.01$) at 46 gm/L of *C. colocynthis* extract. Micronuclei are described as chromatin-containing structures in

the cytoplasm surrounded by a membrane originate from chromosome fragments or whole chromosome lagging at anaphase[37,38].

The microscopic study recorded an extensive cell death (Pyknosis), which is the irreversible condensation of chromatin in the nucleus of a cell undergoing necrosis or apoptosis. It is followed by, fragmentation of the nucleus. The nucleus shrinks in size and the chromatin condenses to a solid, structure less mass. Gupta, *et al*[39] obtained similar result. The dividing cells were inhibited at non-proliferative G0/G1 phase and cells at proliferative S phase obviously decreased by inhibition the expression of the cell cycle`s protein. These changes could lead to increasing apoptosis rate. Meantime, the phenomenon of the increasing cell apoptosis was proved by morphology, which was the condensing and pyknosis of cell nucleus with bright staining[40]**.** These results are together strongly support the role of *C. colocynthis* as anti-carcinogenic agent

Mitotic index is use as an indicator of cell proliferation biomarkers, which measures the proportion of cells in the mitotic phase of the cell cycle. Hence, the decrease in the mitotic index of *A. cepa* meristematic cells could be interpreted as cellular death. Mitotic index was analyzed in this study to determine the genotoxicity of *C. colocynthis* extract treatment on *A. cepa*. After treatment of meristem cells of *A. cepa* root tips with extracts of *C. colocynthis* showed decreased in mitotic index with increasing concentration and duration time of treatments. There were significant differences ($p < 0.01$) in mitotic index at high concentration (46 and 92 gm/L) of *C. colocynthis* compered with control (Table 2). The depressive effect of mitotic index suggests that *C. colocynthis* extract had some effects on preventing cells from proceeding into prophase, the arrest of one or more mitotic phases, or the slowing of the rate of cell progression through mitosis[41]. This is induction that induce of molecular change in the genetic material, suggesting either DNA lesion, or interference with cell cycle[42]. The change of mitotic phases may be resulted by linked or intercalated the one of component of *C. colocynthis*with proteins (histones) or nucleotides of DNA in interphase[43].

Total soluble protein level test of seedlings and DNA extraction:

Soluble protein content in organisms, an important indicator of reversible and irreversible changes in metabolism, is known to respond to a wide variety of stressors such as natural and xenobiotic[44] **.**In this experiment, the observation of the DNA quantities and total soluble protein content of root tips in *A. cepa* seedlings were significant decrease in all treatments compared with the control, that suggesting an inverse relationship with *C. colocynthis* concentration, indicating its potential use as a biomarker. Likewise, the

measure of some parameters at the population level facilitates the interpretation of the data at the molecular level. For example, a significant reduction in root growth of *A. cepa* seedlings correlated with a significant change in RAPD profiles (Figure 6), suggesting that the extent of DNA damage may be serious in the majority of the cells in root tips of *A. cepa* seedlings. These results are consistent with the results obtained by[45].

RAPD profile:

After suitable optimization of the PCR conditions, and the judicious choice of oligonucleotide primers for each species-specific DNA template, reproducible DNA profiles have been generated in terms of number of bands. Product yield and clarity of the profiles from a range of plants, aquatic invertebrates and bacterial species and successfully used to detect DNA damage induced by genotoxic agents[46]. Previous studies have shown that changes in DNA fingerprint (i.e. band patterns) observed reflect DNA alterations in genome from single base changes (point mutations) to complex chromosomal rearrangements[47] and that DNA fingerprinting offers a useful biomarker assay in genotoxicity[48].

Likewise, in the present study, DNA damage induced by *C. colocynthis* extract was reflected by changes in RAPD profiles such as variation in band intensity, disappearance of bands, and appearance of new PCR products occurred in the profiles (Figure 6 and Table 4). These results indicated that genomic template stability in *A. cepa* seedlings was significantly affected by *C. colocynthis*. Modifications of band intensity and lost bands are likely to be due to one or a combination of the following events: (1) changes in oligonucleotide priming sites due mainly to genomic rearrangements and less likely to point mutations and DNA damage in the primer binding sites (because the binding site is only 10 base long whereas genomic rearrangements occur in much longer fragments, e.g. several kb), and (2) interactions of DNA polymerase in *A. cepa* seedlings with damaged DNA. These events could act to block or reduce polymerization of DNA in the PCR product[49]. Appearance of new PCR products occurred because some oligonucleotide priming sites could become accessible to oligonucleotide primers after structural change, or because some changes in DNA sequence have occurred due to mutations (resulting in new annealing events), and/or large deletions (bringing two pre-existing annealing sites closer)[25]. This observation gives good evidence to the ability of *C. colocynthis* extract to induce molecular change in meristem cells of *A. cepa* seedlings. Similar results obtained by[50] in their study to evaluate genotoxic potential of *Thermopsis turcica* aqueous extracts.

Moreover, study of [51] for investigation of the anti-genotoxic potential of methanol and ethanol extracted leaf sap of fenugreek on *Allium cepa* root tip cells by RAPD assay.

CONCLUSION

The obtained results strongly suggest that the leaves extract of *Citrullus colocynthis* is a clastogenic, mutagenic at high dose and anti-carcinogenic agent probably at small dose. For the safe use of this plant in a folk treatment, it needs to further scientific studies on its effects as anti-cancer agent and determine of the effective and safe doses.

REFERENCES

1. Ardekani MR, Rahimi R, Javadi B, Abdi LKhanavi. M. Relationship between temperaments of medicinal plants and their major chemical compounds. J Tradi. Chin Med. 2011; 31 (1): 27–31.
2. Rahimi R, Amin G, Ardekani MR, A review on *Citrullus colocynthis* Schrad.: from traditional Iranian medicine to modern phytotherapy . J Altern Comple. Med.2012;18(6):551-4.
3. Shafaei H, Esmaeili A, Rad H, Delazar A, Behjati M. *Citrullus colocynthis* as a medicinal or poisonous plant: a revised fact. JMPR,2012; 6(35): 4922–4927.
4. Daradka H, Almasad MM, Qazan WS,El-Banna NM. Samara OH. Hypolipidaemic effects of *Citrullus colocynthis L.* in rabbits. Pakis J Bio Scie.2007;10(16):2768–2771.
5. Rahbar AR, Nabipour I, The hypolipidemic effect of *Citrullus colocynthis* on patients with hyperlipidemia.Pak J Biol Sci .2010; 13 (24):1202-7.
6. Huseini HF, Darvishzadeh F, Heshmat R, Jafariazar Z et al. The clinical investigation of *Citrullus colocynthis (L.)* schrad fruit in treatment of Type II diabetic patients: a randomized, double blind, placebo-controlled clinical trial. Phytother Res.2009; 23(8):1186-9.
7. Marzouk B, Marzouk Z, Haloui E, Fenina N, Bouraoui A. et al. Screening of analgesic and anti-inflammatory activities of *Citrullus colocynthis* from southern Tunisia. J Ethnopharmacol.2010; 128(1): 15–19.
8. Dhanotia R, Chauhan N, Saraf D, Dixit V. Effect of *Citrullus colocynthis* Schrad fruits on testosterone-induced alopecia. Natu Produ Res.2009; 16:1–12.
9. Tannin-Spitz T. Bergman M, Grossman S. *Cucurbitacin glucosides*: antioxidant and free-radical scavenging activities. Biochem Biophy Res Commu.2007;364(1): 181–186.

10. Dehghani F, Panjehshahin M. RThe toxic effect of alcoholic extract of *Citrullus colocynthis* on rat liver. Iran J Pharm and Therapeu,2006, 5 (2):117–119.
11. Jouad H. Haloui M, Rhiouani H, El Hilaly J, Eddouks M. Ethnobotanical survey of medicinal plants used for the treatment of diabetes, cardiac and renal diseases in the North centre region of Morocco (Fez-Boulemane). J Ethnopharmacol. 2001;77 (2-3): 175–182.
12. Elawad AA, Abdel Bari EM, Mahmoud OM, Adam SE. The effect of *Citrullus colocynthis* on sheep. Vet Hum Toxicol..1984;26(6):481-5.
13. Bakhiet AO, Adam S,An estimation of *Citrullus colocynthis* toxicity for chicks. Vet. Hum. *Toxicol*.1995; 37: 356-359
14. Al-Faraj S. Hemorrhagic colitis induced by *Citrullus colocynthis*. Ann Trop Med Parasitol.1995;2: 15-19
15. Berrut C, Bisetti A, Widgren S, Tissot JD, Loizeau E. Pseudomembranous colitis caused by the ingestion of *colocynth*. Schweiz Med Wochenschr. 1987; 117(4):135-8.
16. Goldfain D, Lavergne A, Galian A, Chauveinc L, Prudhomme F. Peculiaracute toxic colitis after ingestion of *colocynth*: a clinicopathological study of three cases. Gut.1989;30(10):1412-8.
17. Javadzadeh HR, Davoudi A, Davoudi F, Valizadegan G, Goodarzi H. Citrullus colocynthis as the Cause of Acute Rectorrhagia. Case Rep Emerg, Med.2013; 652192.
18. Migahid MA, Hammouda MA. Flora of Saudi Arabia. Riyad University, 1974; P:574
19. Fiskesjo G. The *Allium* test as a standard in environmental monitoring. Hereditas.1985; 102: 99-112.
20. Vanya P,KalchevaA.P, DragoevaK.N, KalchevD, Enchev D. Cytotoxic and genotoxic effects of Br-containing oxaphosphole on *Allium cepa* L. root tip cells and mouse bone marrow cells. Genet Mol Biol.2009; 32(2): 389–393
21. Qari SH. Assessment of the potential genotoxic risk and antigenotoxic effect of *Cassia senna* L. Leaves aqueous extract using in vitro assays. Egypt. J. Biotechnolo.2008; (30): 101-116.
22. Bradford M. Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. Analytic. Bioche.1976; 72, 248-254
23. Scott D, Galloway SM, Marshall RR, Ishidate M, Brusick D. Genotoxicity under Extreme Gultue Conditions.A report from ICPEMC Task Group 9. Mutat. Res.1991; 257, 147 – 204.
24. Williams J, Kubelik AR, Livak KJ. DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucleic Acid Research.1990;18: 6531–6535.

25. Atienzar FA, Conradi M, Evenden AJ, Jha AN,Depledge MH. Qualitative assessment of genotoxicity using random amplified polymorphic DNA: comparison of genomic template stability with key fitness parameters in *Daphnia magna* exposed to benzo[a]pyrene. Environ. Toxicol. Chem. 1999;18: 2275–2282
26. Luceri C, De Filippo C, Caderni G, Gambacciani L, Salvadori M. Detection of somatic DNA alterations in azoxymethane-induced F344 rat colon tumors by random amplified polymorphic DNA analysis. Carcinogenesis 2000;21: 1753–1756
27. Magdaleno A, Gavensky M, Fassiano AV, Ríos de Molina MC. et al. Phytotoxicity and genotoxicity assessment of *imazethapyr* herbicide using a battery of bioassays. Environ Sci Pollut Res Int.2015; Aug 7.
28. Qari SH, Detection of DNA Damage in *Allium cepa* Root Cells After Exposure to Carbofuran Using RAPD Assay. J. UQU – Appl. Scie.2009; 1(1): 45-57
29. Rank J, Nielsen MH. *Allium cepa* anaphase-telophase root tip chromosome aberration assay on N-Methyl-N-nitrosourea, maleic hydrazide, sodium azide, and ethyl methanesulfonate. Mutat. Res. Genet. Toxicol. Environ. Mutagen.1997; 390: 121–127.
30. Carita R, Marin-Morales MA. Induction of chromosome aberrations in the *Alliumcepa* test system caused by the exposure of seeds to industrial effluents contaminated with azo dyes. Chemosphere 2008;72: 722–725.
31. Leme DM, Marin-Morales MA. *Allium cepa* test in environmental monitoring: A review on its application. Mutat. Res. Rev. Mutat. Res.2009;682: 71–81.
32. Shehab A, Adam ZM. Cytological effects of medicinal plants in Qatar III. Mitotic effect of water extract of Anastatica hierochuntico L. on *Allium cepa*. Cytologia 1983; 48: 343–348
33. Baeshin NA, Al-Ahmadi M. Assaying single and combined genotoxicity of Calotropis procera Ati latex and Chlorcyrin in root merstems of *Allium cepa* and *Vicia faba*. Tanta Univ., Egyp. ICBS.2004; 3(1): 148-199.
34. Baeshin NA, Qari SH. Combined genotoxic and Cytotoxic effects of cadmium chloride and Carbofuran in root meristems of *Vicia faba*. Saudi, J. Bio. Sci.2003; 10(1):107-119.
35. Odeigah P, Nurudeen O, Amund O. Genotoxicity of oil field wastewater in Nigeria. Hereditas.1997; 126: 161–167.

36. Baeshin NA, El-Seoudy AA, Al-Ahmadi MA. Single and combined genotoxic effects of *Furadan* and Sequestrene (138 Fe 100 SG) in root meristems of *Allium cepa*. Proc. Third International Symposium.1999.
37. Fenech M. The in vitro micronucleus technique. Mutat. Res./Fundam. Mol. Mech. Mutagen.2000;455: 81-95.
38. Yi H. and Meng Z. Genotoxicity of hydrated sulfur dioxide on root tips of *Allium sativum* and *Vicia faba*. Mutat. Res. Genet. Toxicol. Environ. Mutagen.2003; 537: 109–114
39. Gupta K, Gaumat S, Mishra K. Studies on phyto-genotoxic assessment of tannery effluent and chromium on *Allium cepa*. J Environ Biol.2012;33(3):557-63.
40. Ai-Hong J, Zhao-Lian W. Molecular mechanism of increased sensitivity of cisplatin to ovarian cancer by inhibition of microRNA-23a expression. Int J Clin Exp Med.2015; 8(8): 13329–13334.
41. Christopher HB. Kapoor MB. The cytogenetic effects of sodium salicylate on the root meristem cells of *Allium sativa L*. Cytologia.1988; 54:203–209
42. Sabir JS. Qari SH, Baeshin NA. Cytotoxic and genotoxic effects of Carbofuran in root meristems of *Vicia fava.* Proceeding of the International Congress on Molecular Genetics.1998; 1:87-94.
43. Jacobson KB. Turner JE. The interaction of cadmium and certain other metal ions with proteins and nuclic acids. Toxicology.1980;16:137
44. Singh R, Tiwari R, Datta D. Detection of leaf rust resistance genes Lr9 and Lr10 in wheat (*Triticum aestivum*) by PCR based STS markers. Acta Phytopathologica et Entomologica Hungarica.2003; 38(3-4): 245-249
45. Morita H, Awang K, Hadi AH, Takeya K. Itokawa H. et al. Conformational analysis of rhazinilam and three-dimensional quantitative structure-activity relationships of rhazinilam analogues. Bioorg Med Chem Lett. 2005;15(4):1045-50.
46. Grayson TH. Cooper LF. Atienzar FA. Knowles MR. Gilpin ML. Molecular differentiation of *Renibacterium salmoninarum* isolates from worldwide locations. Appl Environ Microbiol. 1999;65(3):961-8.
47. Atienzar FA, Venier P, Jha AN, Depledge MH. Evaluation of the random amplified polymorphic DNA (RAPD) assay for the detection of DNA damage and mutations. Mutat Res. 2002; 26;521:(1-2):151-63.
48. Savva D. Use of DNA fingerprinting to detect genotoxic effects. Ecotoxi Enviro. Safety.1998; 41: 103–106.

49. Nelson DR, Koymans L, Kamataki T, Stegeman JJ, Feyereisen R. et al. P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature.Pharmacogenetics. 1996; 6(1):1-42.
50. Ciğerci İH, Cenkci S, Kargıoğlu M, Konuk M. Genotoxicity of Thermopsis turcica on *Allium cepa* L. roots revealed by alkaline comet and random amplified polymorphic DNA assays. Cytotechnology. 2014; 1:31-38
51. Mekki L. Genoprotectivity of methanol and ethanol extracted leaf sap of *Trigonella foenum-graecum* in *Allium cepa* root assay. Acta Biol Hung. 2014; 65(1):85-95.