

CYTOGENETIC EFFECTS OF THE CRUDE SAPONINS OF *Yucca elephantipes* L. LEAVES ON THE *Allium cepa* L. ROOT TIPS MITOSIS.

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ABSTRACT

This study was aimed to investigate cytogenetic effects of the crude saponins of *Yucca elephantipes* leaves on mitosis. The root tips of *Allium cepa* L. were used as plant test system *in vivo*. Root tips of *A. cepa* were tested for four hours with four concentrations of the crude saponins (0.00, 6.25, 12.5, 25 or 50 mg/ml). This study were included some cytogenetic diagnosis included mitotic index, phase index, and chromosomal aberration. Results showed that saponins reduced the mitotic index (MI) to less than 50%, specifically when treated with 12.5, 25 or 50 mg/ml mitotic index reached 25.48, 17.98, 18.81% respectively, this reduction was considered toxic and sub lethal. Saponins arrested chromosomes at metaphase (c-metaphase) up to 100% at 6.25 mg/ml and chromosomal aberrations including micronuclei and nuclear lesion (chromatin lesion) in prophase and interphase at the concentrations 25 and 50 mg/l recorded 1.30-8.30%.

Key words: c-metaphase, mitotic index, cell division, *in vivo*, micronuclei.

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التأثير الوراثي الخلوي للصابونيات الخام لأوراق نبات اليكة *Yucca elephantipes* L. على الانقسام الخلوي في خلايا القمم النامية لجذور البصل *Allium cepa* L.

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باحثة

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مدرس

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المستخلص

كان الهدف من الدراسة التحري عن التأثيرات الوراثية للصابونيات الخام في اوراق نبات اليكة *Yucca elephantipes* على الانقسام الخلوي. عوملت خلايا القمة النامية لجذور البصل *Allium cepa* لمدة اربع ساعات باربعة تراكيز من الصابونيات الخام, اضافة الى المقارنة (0.00, 6.25, 12.5, 25, 50 ملغم/مل). تضمنت الدراسة بعض المعايير الوراثية الخلوية, مثل: دليل الانقسام الخلوي, دليل الطور, ودليل الزيغ الكروموسومي او الحالات الشاذة. ادت المعاملة بالصابونيات الى خفض دليل الانقسام الخلوي كنسبة مئوية من المقارنة الى دون 50% وخاصة عند المعاملة بالتراكيز 50, 25, 12.5 ملغم/ملاذ وصل دليل الانقسام الى 17.98, 25.48, 18.81% بالتتابع, مما يدل على تأثيره السمي او شبه القاتل. كذلك اظهرت الصابونيات قابلية تطفيرية اذ ظهرت حالات مختلفة من الزيغ الكروموسومي متمثلة بالاستوائي المتوقف بلغت اعلى نسبة لها 100% عند التركيز 6.25 ملغم/مل وبعض حالات الزيغ الكروموسومي مثل الانوية الصغيرة و الافة النووية (تحلل الكروماتين) في الطورين الابتدائي والبيني وبنسبة عالية عند التركيزين 25 و 50 ملغم/مل وبنسبة مئوية 1.30-8.30% بالتتابع.

الكلمات المفتاحية: استوائي متوقف, دليل الانقسام الخيطي, انقسام الخلية, داخل الجسم الحي, النويات الصغيرة.

INTRODUCTION

Some plants and their natural compounds are the main sources of many medicinal uses in modern medicine. Plants have been used in medicine for years, as well as being a major source of food and therapeutic products. World Health Organization (WHO) has shown that more than 80% of the world's population still uses many medical plants in treatment (10, 19). Many scientists are interested in their studies on many natural compounds that are possible to protectors against many diseases such as cancer. The spineless yucca, *Y. elephantipes* belongs to monocotyledons Agavaceae family, grows widely in North and Central America and México. It is also known as giant yucca, common yucca or *Y. guatemalensis* (26). The presence of saponins in *Y. elephantipes* was reported, some of which have been isolated and identified (27). The saponins are class of natural products which are structurally constructed of aglycone (tri-terpene or steroid) and sugars (hexose and/or uronic acid). The 'saponin' nominated from soap as its containing plants agitated in water form soapy lather. Saponins were widely distributed in many plants and are relatively widespread in our foodstuffs and herbal preparations. Saponins traditionally used as a natural detergent (17). In addition to this physical property, plant-derived triterpenoid and steroidal saponins have historically received a number of industrial and commercial applications ranging from their use as sources of raw materials for production of steroid hormones in the pharmaceutical industry, to their use as food additives and as ingredients in photographic emulsions, fire extinguishers and other industrial applications which take advantage of their generally non-ionic surfactant properties (7). *Yucca* is also rich sources of polyphenolics, including resveratrol and a number of other stilbenes (yuccaols A, B, C, D, and E). These have anti-inflammatory activity. They are inhibitors to the nuclear transcription factor NFkappaB. NFkB stimulates the synthesis of inducible nitric oxide synthase (iNOS), which causes the formation of the inflammatory agent nitric oxide. Yucca phenolics are also anti-oxidants and free-radical scavengers, which may aid in suppressing reactive oxygen species that

stimulate inflammatory responses (9). Several plants extract were studied in the effect of mitotic division and the study of chromosomes structure and diagnosed the causes of some chromosomal aberration for which it was diagnosed as a cell-disruptive agent such as colchicine, vinca alkaloid, and Taxol, which were known to inhibit cell division and tumor cell growth, and were commonly used in cancer treatment (28,2). Cytological analysis with respect to either mitotic or meiotic behavior is considered one of the most dependable indices to estimate the potency of mutagen and sensitivity of plants for different mutagens. The mitotic index (MI) is a reliable predictor of cell proliferation in the tissue. MI assay is used to characterize proliferating cells in mitosis and the total number of cells. The mitotic index can be worked out from a slide; even with light microscopy. The MI is a cytogenetic test that used *in vivo* and *in vitro* for the examination of genotoxic effects of a compound over a short period and there are considerable variations in the mutagenic effect of agents in different environments (15). The types of rearrangements are deletions, fusions, and interchanges as well as changes in ploidy level. These aberrations cause changes in phenotypic expression of one or more genes. Sometimes changes in chromosome number and DNA content. According to one suggestion, chromatin diminutive before the cells become competent to regenerate. Another theory suggests that chromosome breaks may be induced in culture by the late replication of heterochromatin (18). The degree of cytological aberrations in either mitosis or meiosis is regarded as one of the dependable criteria for estimating the effect of a mutagen. Mutagen induced chromosome aberration is the primary basis of genetic change; therefore, investigations on the mechanism of chromosome breakage, type of aberrations, and their genetic consequences form an integral part of most mutation studies. The most aberrations induced by mutagen are lagging chromosomes, bridges, translocation, and sticky chromosomes. Cytogenetically investigation is one of the best documented experimental proofs for the elucidation of the mode of speciation on different groups of plants (12). Inhibitors, mutants, and

genotoxicity of certain chemical compounds, such as pesticides, chemicals, and others that affecting the genetic system of eukaryote organisms, are testing by using onions (*A. cepa*) system (13,30). Onions contain large chromosomes, in small numbers, contains on a large set of split cells and in different phases. There are several research programs to evaluate the effect of plant extracts on cell division and their potential use in many research programs; therefore, *Y. elephantipes* was selected to use their crude saponins in the current study that was aimed to study the effect of these saponins on the mitotic division in roots tips of *A. cepa* and comparison of their effects with cytotoxicity in terms of the mitotic index and the percentage of abnormal cells.

MATERIALS AND METHODS

Plant collection: This work was carried out in the Laboratory of Biology department, University of Baghdad, Baghdad, Iraq during the period 1/10/2017 - 1/5/2019. Leaves of *Y. elephantipes* plant were obtained from the plants nursery. In November 2017. The studied sample was collected randomly from the plant, taking into account that the plant parts used in the extraction process were free from visible damage and uniform in the size. Leaves were washed well with tap water to remove dust, then cut into small pieces and then placed in the oven for drying at 50-55°C, and then placed in the mill until it became a powder.

Extraction of crude saponins

Crude saponins were extracted from *Y. elephantipes* were extracted by heating the powdered sample (20 g) for 4 h at 55°C with 100 ml of ethanol (20%). The extract was filtered and residue was re-extracted with 200 ml of ethanol (20%). The extract was concentrated on water bath till the volume reduced to 40 ml, which was mixed with 20 ml diethyl ether in a separating funnel. The mixture was shaken vigorously and then the separating funnel was fixed in a stand till the formation of aqueous and diethyl layer. Aqueous portion was collected while the diethyl ether portion was discarded. To the aqueous layer *n*-butanol (60 ml) was added and properly mixed by vigorous shaking. The *n*-butanol extract was treated with 10 ml of 5% NaCl solution. The resultant solution

was concentrated on a water bath and the crude saponins were dried in an oven (31).

Preparation of plant extracts

Stock solutions were prepared by mixing 1 g from the dried saponins with 10 mL distilled water, and then sterilized with the millipore filter (0.22 µm). Then different concentrations of 10 mg/ml were prepared by mixing known volume from the stock solution using distilled water. A series of different extract solutions were prepared at the concentrations 6.25, 12.5, 25 or 50 mg/ml. (3)

Effect of crude saponins on cell division of onion root tips

Onion bulbs were purchased from local market, old roots and dry scales were removed and allowed to grow by placing in flasks containing tap water. When the roots reached 2-3 cm in length, the root meristem was exposed to various concentrations of *yucca* crude saponin at its peak mitotic time. Four concentrations (0.0, 50, 25, 12.5, and 6.25 mg/ml) for four hours were evaluated for their cytotoxic potential by *Allium* assay. Mitotic squash preparations of the root tips as reported by Sharma and Sharma was carried out (24). Each experiment was repeated three times, and at least, five micro glass slides were prepared for each parameter.

Treatment of roots and preparation of slides

Root tips were dissected from treated and non treated onion root tips and subjected to karyotypic studies as follows:

1. Excised roots from the growing bulbs were grown at 25-30 °C in dark, and dissected leaving 1-2 mm of the root tips. Root tips were stabilized in carnoy fluid to study cell division. It included three volumes of absolute ethylene alcohol with one volume of Glacial acetic acid and the preparation is pre-use(22, 14).
2. Root tips were treated with 2-3 drops of 1 N HCl inside vials at 55-60 °C, placed in an oven for 10 min.
3. Root portions were washed with distilled water then transferred to another vial containing acetocarmine stain 2%, placed in an oven for 10 min.
4. Excess stain was removed carefully, then one drop of fresh stain was added on a dot-sized piece of the root tip before placing slide

covers carefully, then root tips were squashed (24)

5. Using a compound light microscope (40X), the meristematic region of the root tip was allocated. Slides were examined at 100X magnification and chromosomes at (various stages of mitosis were seen and photographed using I phone camera.

Mitotic index

The percentage of cells undergoing mitosis in each treatment was determined on the basis of a minimum of 1000 cells. Abnormal dividing cells were calculated according to the equation:

%Mitotic Index (MI) = [Total number of dividing cells /Total number of cells examined] * 100 (4, 20).

% phase Index = [Total number of phase /Total number of dividing cells] * 100

% aberration Index = [Total number of aberrant cells /Total number of dividing cells in the same phase] * 100 (20).

The percentage of control for mitotic index and phase index was calculated in onion roots for treatment with different plant extracts:

1-Percentage of control for Mitotic index = [Mitotic index in each treatment/ Mitotic index in control] * 100

2-Percentage of control for phase index = [Treatment phase index / control phase index] * 100 (1).

Experimental design and statistical analysis

The experiments were designed according to the completed randomized design and three replicates per treatment. The results were analyzed using SPSS program and means were compared to the Duncans multiple range to determine the significant differences among the means of the split index at a probability level $P \leq 0.05$.

RESULTS AND DISCUSSION

The effect of Yucca crude saponins on the cell division of onion root tips *Allium cepa*

Mitotic index

Mitotic index reduced significantly at all saponin concentrations, recording 29.5, 19.16, 7.519, 5.307, and 5.55% for control, 6.25, 12.5, 25, 50 mg/ml) respectively (Figure 1 and Table 1). No significant differences were revealed between control and the treatment with 6.25 mg /ml as shown in Table 1. Mitotic index decreased less than 50% at 6.25, 12.5,

25 and, 50 mg/ml recording 25.48, 17.98, 18.81% respectively (Figure1 and Table 2). Results are in accordance with(6, 23), who explained that substances and extracts that cause a reduction in the mitotic index up to 50% or less have a toxic or semi-lethal effect on the mitotic index can interfere with the stages of division and thus prevent the cell nucleus from entering the mitotic phase, then stops the mitotic division during the interphase phase or by increasing the phase at G2, S phase or inhibits the process of protein and DNA synthesis.

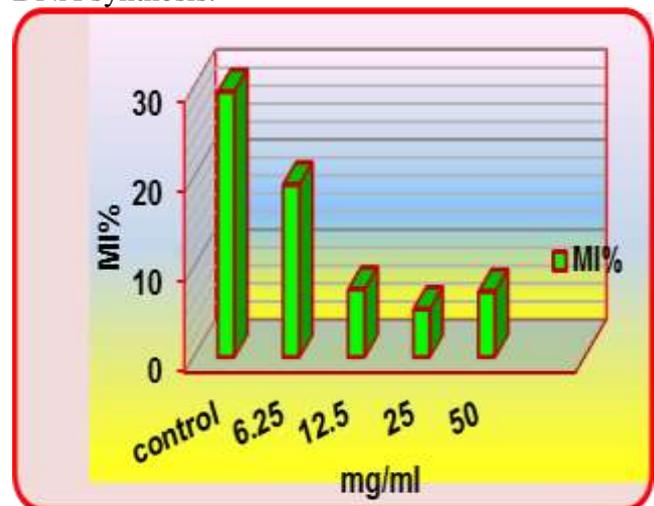


Figure1. Mitotic index% in onion root tips treated with crude saponin of Yucca leaves in different concentrations

Prophase

Prophase index recorded 71.42, 74.08, 80.26, 76.27 and 78.26% in control, 6.25, 12.5, 25, 50 mg/ml respectively as shown in Table 1. The percentage of control increased up to 95.69, 112.38, 106.80, and 109.57% at 6.25, 12.5, 25 and 50 mg/ml respectively (Table 2). Some abnormal cells attributed in this phase such as abnormal cell shape (Figure 2-C).

Metaphase

Metaphase decreased significantly and recorded 5.03, 2.95, 5.05% at 12.5, 25, 50 mg/ml, respectively, compared with control 11.38% (Table 1). The percentage of control decreased in 12.5, 25, and 50 mg/ml recording 44.20, 25.96, and 44.37% respectively as shows in Table 2. Chromosomal aberrations like C-metaphase in metaphase were also noticed. C-metaphase was very frequently seen even at low concentrations of saponin recording 100 and 25% at 6.25 and 12.5 mg/ml respectively. This could be indicate that studied saponins contain

compounds that have anti-microtubules and polymerization of tubulin which form spindle filaments. So chromosomes were failed to complete their alignment in metaphase (25, 8). The effect may due to the mechanisms of nuclear division and then had an effect on the formation and distribution of spindle threads and stop the cellular division (16).

Anaphase and telophase

Anaphase decreased significantly and recorded 3.96, 2.54, 2.98 % at 12.5, 25, 50 mg/ml respectively, compared with control 6.45% (Table 1), while, the percentage of control increased at 6.25 mg/ml reached 101.70% . The percentage of control decreased in 12.5, 25, 50 mg/ml recorded 61.49, 39.48 and 46.30% respectively as shows in Table (2). Telophase in control was 10.73, and no significant differences between control and those treated with 6.25 and 12.5 mg/ml. Significant differences are shown when treated with 25 or 50mg/ml respectively (Table 2). Non-clastogenic aberration was shown in these phases. recording 18.57 and 17.35% respectively (Table 1). The percentage of control increased in 25 and 50 mg/ml recorded 173.11 and 193.45%,

Cytological aberrations

Cytological aberration was observed within cells after the treatment with saponins (c-metaphase, micro nucleated cells, MNCS and nuclear lesion (Chromatin lesion) (Figures 2-A, -B, C, D, E). The *A. cepa* test is considered as the important test *in vivo* where the roots grow in direct contact with the substance of interest enabling possible damage to DNA. In this study this test enables to assessment of different genetic endpoints which occur as a results from exposure to Saponin. Saponins caused significant inhibitions of MI in *A. cepa* meristem cells and induced nuclear abnormalities and micro nucleated cells (MNCs). The decreases in the MI as the concentration of crude saponin increased. The

changes in MI of *A. cepa* cells are indicators of cytotoxic and genotoxic potential activity of saponin. At lower concentrations of saponins, non-clastogenic aberrations were observed. Aneugenic effects like abnormal cell shape (Figure 2C), C-metaphase (Figure-2 A and B). At higher concentrations of saponins, a significant increase in the frequency and diversity of aberrations were observed such as nuclear lesions (Figure-2 D) in prophase and interphase were the highly frequent aberration. Proportional increases in the incidence of micronuclei (MN) were also observed at higher concentration. The results showed that higher concentrations were able to inhibit cell division significantly (Figure 1), indicating the toxicity of the tested concentration on *Allium* root tip cells (5). Saponins interfere with the replication of cellular DNA and they prevent the proliferation of the cells. Chromosomal aberrations and chromatid aberrations were noticed in both extracts. Single and multiple lesions were the most frequent aberrations in prophase and interphas cells. Lesions are the results of inhibition in S phase of the cell cycle where duplication of DNA strands happens (11). MN is the name given to the small nucleus that forms whenever a chromosome or a fragment of a chromosome is not incorporated into one of the daughter nuclei during cell division. It is usually a sign of genotoxic events and chromosomal instability. MN was frequently observed at higher concentrations of saponin which clearly indicates the presence of compounds that can stop cell cycle checkpoint genes (21). Wang *et al.*, (29) found that tea flower saponin (TFS) had biological properties, they evaluated the anti cancer effects of (TFS) using human ovarian cancer cell line, TFS induced S phase arrest, furthermore S phase arrest was associated with a Chk2-Cdc25A DNA damage response.

Table 1. Mitotic index, phases, and percentage of abnormality in *A. cepa* roots at different concentrations of crude saponin after four hours

Concentration mg/ml	MI%	Phase index % \pm S.D				Chromosomal aberration %		
		Prophase	Metaphase	Anaphase	Telophase	C-metaphase	Nuclear-lesion	Micro nuclei
Control	29.50	68.57	11.38	6.45	10.73			
	a \pm	\pm	\pm	\pm	\pm			
	6.79	10.00	2.85	1.53	2.45			
6.25	19.17	74.09	8.29	6.55	11.05	100	3.17	
	\pm	\pm	\pm	\pm	\pm			
	2.92	5.88	2.11	1.55	2.31			
12.5	7.52	80.26	5.03	4.29	7.85	65.55	1.30	
	\pm	\pm	\pm	\pm	\pm			
	3.24	5.52	1.31	0.92	3.53			
25	5.31	76.28	2.95	2.64	18.57		8.38	5.94
	\pm	\pm	\pm	\pm	\pm			
	1.86	1.00	0.25	0.43	1.16			
50	7.27	78.35	5.05	2.99	17.30		10.4	13.3
	\pm	\pm	\pm	\pm	\pm			
	3.07	2.34	1.94	0.43	2.01			
LSD P \leq 0.05	12.54	18.46	6.00	3.44	7.61			

Table 2. Mitotic division (%control) and the phases in the onion root tips treated with different concentrations of crude saponin for four hours

Concentration mg/ml	MI%	Prophase%	Metaphase%	Anaphase%	Telophase %
6.25	64.07	95.69	72.84	101.70	102.98
12.25	25.48	112.38	44.20	61.49	73.15
25	17.98	106.80	25.96	39.48	173.11
50	18.81	109.56	44.37	46.30	139.45

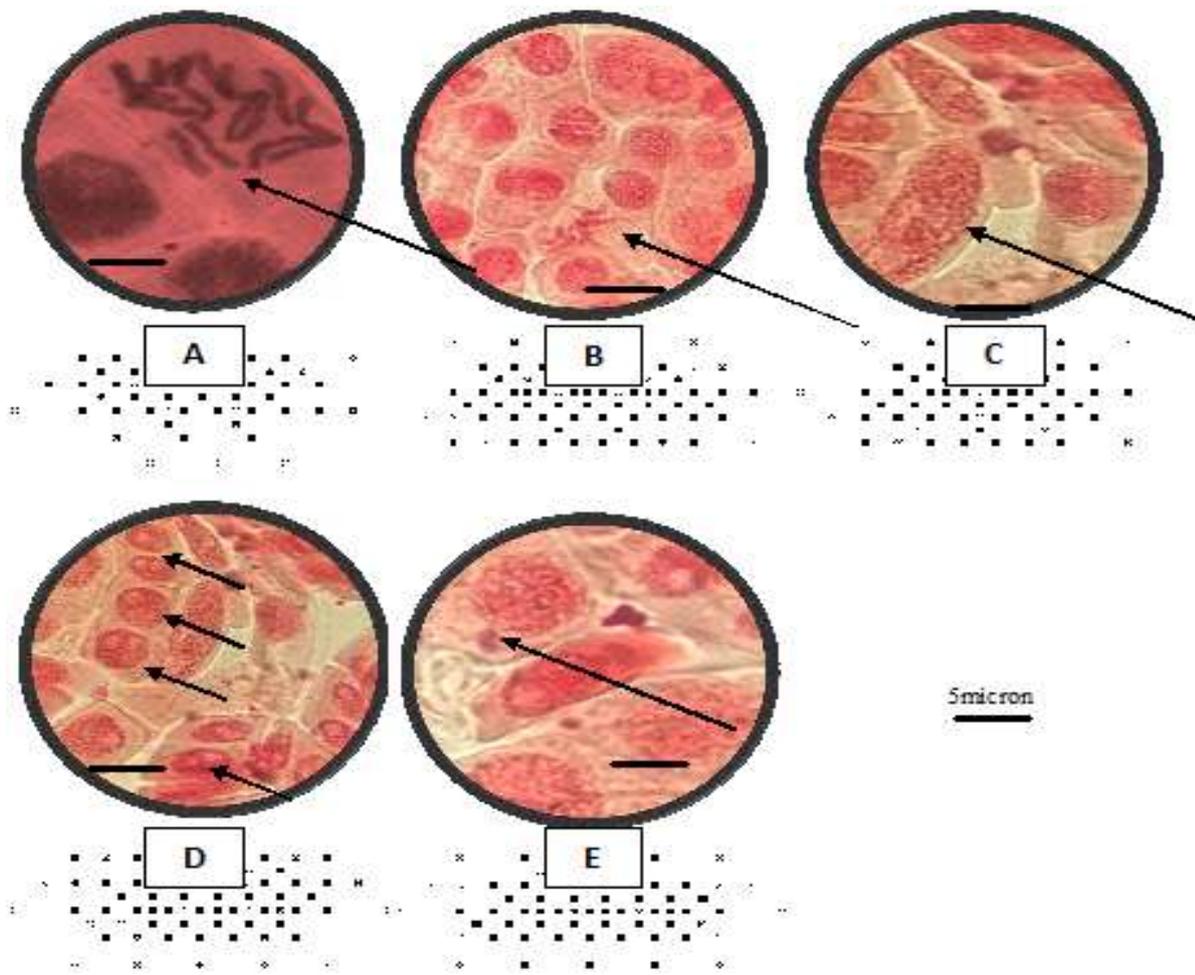


Figure 2. Cytological aberrations in root tip of meristematic cells in *Allium cepa* treated with saponin for four hours, A, B: C- metaphase, C: abnormal cell shape, D: nuclear lesion, E: Micronuclei (MN). X=1000 — 5 micron

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