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Evaluation of Cytotoxicity and Genotoxicity of *Juniperus phoenicea* Leaf Extracts with Allium Test

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Abstract \

Juniperus phoenicea has been used for years in folk medicine for its anti-inflammatory and antioxidants. In this study, cytotoxic and genotoxic effects of *Juniperus phoenicea* leaf extracts on the root meristem cells of *Allium cepa* have been examined. Onion bulbs were exposed to different concentrations of *Juniperus phoenicea* and then submitted to microscopic analysis using acetocarmine stain. Distilled water was used as a negative control. The results showed that, under the testing conditions, the mitotic index (MI) of the onion roots submitted to *J. phoenicea* leaf extracts treatment did not differ significantly from the negative control, which suggests that the *J. phoenicea* is not cytotoxic. Low incidence of chromosome aberrations in the cells treated with *J. phoenicea* leaf extracts was also observed, indicating that the *J. phoenicea* does not have genotoxic effect either. The mitotic index and the chromosome aberration frequency responded to the *J. phoenicea* concentration, requiring more studies to evaluate the dosage effect on genotoxicity Using Spectrophotometric analysis of DNA concentration and Gel electrophoresis.

Key words: Cytotoxicity, Genotoxicity, *Juniperus phoenicea*, *Allium cepa*.

Introduction\

Medicinal plants and indeed plants in general, synthesize toxic substances, which in nature act as a defense against infections, insects and herbivores, but which often affect the organisms that feed on them (35). Reports of unwanted side effects and potential toxicity of phytomedicines have been on the increase in recent years (11, 30, 4, 22, 25, 24). Based on their long-term use by humans, one might expect herbs used in traditional medicine to have low toxicity. It is known that green plants in general are a primary source of antimutagens as well as natural toxic agents (31), and many plants contain cytotoxic and genotoxic substances. Recent investigations have revealed that many plants used as food or in traditional medicine have mutagenic effects and cytotoxic and genotoxic effects in vitro and in vivo assays (19&5). This raises concern about the potential mutagenic or genotoxic hazards resulting from the long-term use of such plants. Many plants contain mutagenic and/or carcinogenic substances (3&14). In the traditional system of medicine different Juniperus species have been used to treat various infectious and inflammatory diseases (2). Juniperus species are considered to help in maintaining good health (13). The phytochemical investigation of the methanolic extract of *J. phoenicea* revealed the presence of four flavonoid compounds namely, myricitrin, quercetin, cosmosin, quercitrin and two phenolic compounds; ρ-coumaric acid and caffeic acid (18, 10, 1). Traditionally, *J. phoenicea* had been taken by mouth to treat conditions of the gastrointestinal tract, such as gas indigestion and poor appetite (7). Their oils also help to increase the flow of digestive fluids, improve digestion and eliminate gas and stomach cramping (36). Currently, there is no published data on the cytotoxicity and genotoxicity of *J. phoenicea* leaf extracts. One way to evaluate the toxicity of natural extracts of natural extracts of plants and/or medicinal plants is the *Allium cepa* assay. This in vitro test is very useful as a first tier analysis of cytotoxicity and genotoxicity, because of the simplicity, low relative cost, versatility and minimum laboratory facilities required for its performance (21). The mitotic index (MI), characterized by the total number of cells in the cell cycle, has been a parameter to assess the cytotoxicity of several agents. The cytotoxicity level of an agent can be determined by an increase or decrease in the mitotic index, mitotic index significantly lower than the negative control can indicate alterations deriving from the chemical action in the growth and development of the exposed organism. On the other hand, MIs higher than the negative control result from an increase in cell division, which can be

harmful to the cells and lead to disorderly cell proliferation and even to the formation of tumor tissues (26), in the present study was conducted to evaluation of Cytotoxicity and Genotoxicity of *J. phoenicea* Leaf Extracts with Allium Test.

Materials and Methods\

Plant materials: The fresh *J. phoenicea* were collected during from Al-Marj plain in Al- Jabal Al-Khdar, Libya. The study was carried out at the labs of Scientific Research, University, Department of Botany, Faculty of Arts and Science - Al marj, Benghazi University, Libya during the period of June. 2018- September, 2019.

Preparation of the Aqueous Extracts of *Juniperus phoenicea* Leave: The extracts were prepared according to the traditional use in Libya and we used in this study crude extracts of *J. phoenicea* leaves. Studying with crude extracts is appropriate because traditional medicinal herbs are generally used as crude extracts. The collected leaves were then dried in the open air for fifteen days and conserved for extraction and subsequently milled to a fine powder by ground into fine powder using a kitchen blender. The powder was placed in small plastic bags (100 g each) and stored at 4°C until use. The extract was prepared by boiling 10 g powdered plant material mixed with 100 ml distilled water for covered beaker (10% stock solution) for 5 min and, cooled to room temperature for 10 min. Thereafter, the extract was filtered through a filter paper (ISO Lab. Quantitative Filter Paper) to remove particulate matter. Stock solution was diluted with distilled water to 2.5 mg/ml, 5 mg/ml, and 10 mg/ml concentrations. Fresh extract was prepared daily for each experiment, just before administration.

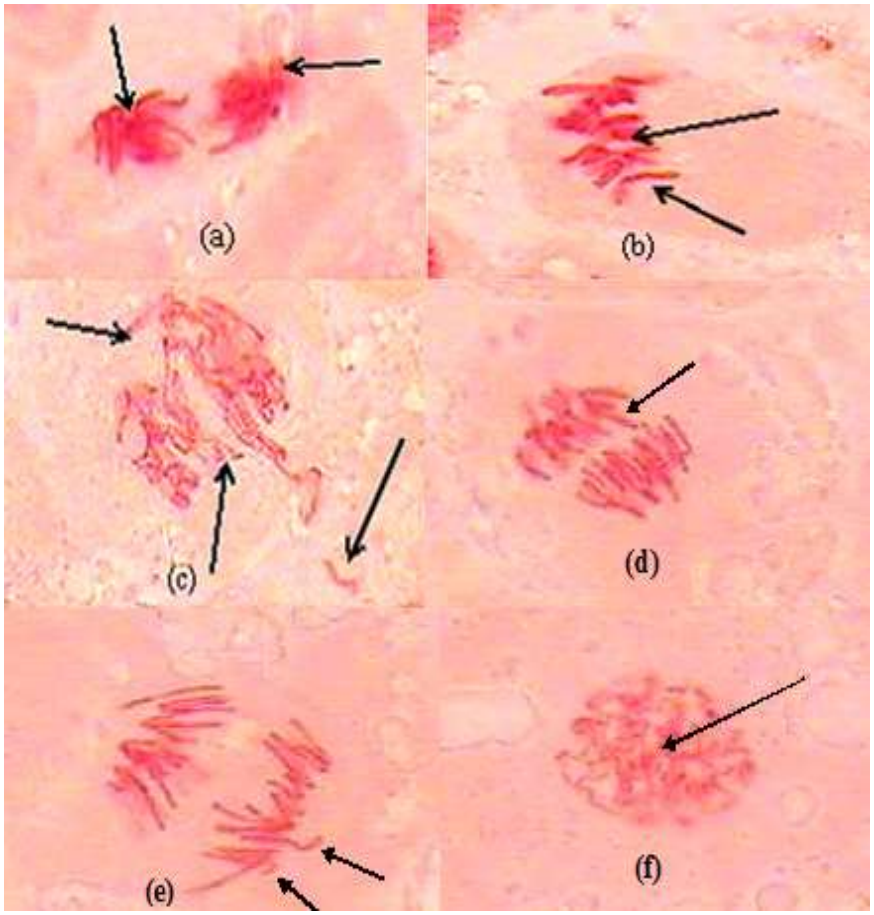
Allium Test: Experiments were planned as per standard protocol for Allium test (15, 17, 23). Onion bulbs (*Allium cepa*, 2n=16, Family Amaryllidaceae). They were sun dried for two weeks so as to reduce the moisture content and to facilitate root growth. For each herb/recipe extract, three concentrations (v/v) viz: 2.5, 5, 10 mg/ml. Two exposure periods of 2 h (Group I) and 24 h (Group II) were utilized in this study. At each concentration for each group and the control, 5 onion bulbs were grown in tap water in 100 ml beakers (27±1 o C) for 2 days in the dark. After 48 h of root sprouting, the bulbs were transferred into the respective concentration of the extracts and allowed to grow for time intervals of 2 h (Group I) and 24 h (Group II) respectively. Samples from the experimental and the control groups were changed every 24 h. At the end of the treatment period for each group, root tips were cut and fixed in ethanol: glacial acetic acid (3:1 v/v). Thereafter, these were

hydrolyzed in 1N Hcl at 60 °C for 5 min after which they were washed in distilled water. Two root tips were then squashed on a microscope slide and stained with acetocarmine for 10 min. Excess stain was removed and cover slips were carefully placed on the smear. The cover slips were sealed on the slides with clear fingernail polish as suggested by (17). Six slides were prepared for each concentration and the control out of which four (at 1000 cells / slide) were scored for induction of aberrant cells at x1000 magnification. The mitotic index was calculated as the number of dividing cells per total cells scored at each concentration. The frequency of aberrant cells (%) was calculated based on the number of aberrant cells per total cells scored for each concentration of the extract (6). The mitotic inhibition was obtained as follows: Mitotic inhibition = (mitotic index in control – mitotic index in treated group) ×100 / (mitotic index in control).

Statistical Analysis: Statistical analyses were performed using the SPSS 11.5 software package programme. Data on mitotic index and chromosomal aberrations were compared using analysis of variance (ANOVA) to confirm the variability of the data and validity of results. Differences between corresponding controls and exposure treatments were considered statistically significant at $P < .05$.

Results\

The results showed that, under the testing conditions, the mitotic index (MI) of the onion roots submitted to *J. phoenicea* leaf extracts treatment did not differ significantly from the negative control after 2 and 24 h exposure period. This indicates that *J. phoenicea* leaf extracts does not have a cytotoxic effect on *A. cepa* cells. The mitotic index (MI) increased progressively according to the increase in that *J. phoenicea* leaf extracts concentration. Chromosome aberrations were observed in very low frequency in all stages of mitosis. Table 1 shows the frequency of chromosome aberration in each treatment, it was possible to observe a decrease of different abnormalities as the *J. phoenicea* leaf extracts concentration increased. In Allium test, a weak toxic effect of *J. phoenicea* leaf extract was observed. A statistically significant decrease in total aberrant cells ($P < .05$) aberrant cells include chromosome fragments, stickiness, polar deviation, Anaphase bridges, Lagging chromosome and Early condensation in prophase as compared with the negative control (Table 1), were also observed (Figures 1(a), (b), (c),(d),(e) and (f)).



Figures 1: Chromosomal aberrations examined in mitotic phases of *A. cepa* root tip cells. (a) Polar deviations, (b) Sticky metaphase, (c) Chromosome fragments, (d) Anaphase bridges,(e) Lagging chromosome and (f) Early condensation in prophase.

Table 1. Cytogenetic analysis of *A. cepa* root tips exposed to different concentration of *Juniperus phoenicea.*

Treatments		Cytotoxicity			Genotoxicity						
Time	Concentrations (mg/ml)	Total cells	Dividing cells	MI (%) ± SD	Polar deviations	Sticky metaphase	Chromosome fragments	Anaphase bridges	Lagging chromosome	Early condensation in prophase	Aberrant cells %
2h	control	4050	1170	28.88 ± 1.43	0	0	0	0	0	0	0
	2.5 mg/ml	4015	1200	29.89 ± 1.88	1	2	1	0	2	1	0.17
	5 mg/ml	4055	1115	27.49 ± 1.56	0	1	3	1	0	1	0.15
	10 mg/ml	4010	1150	28.67 ± 1.82	0	1	0	1	2	2	0.15
24 h	control	3794	950	25.03 ± 1.02	0	0	0	0	0	0	0
	2.5 mg/ml	3789	915	24.15 ± 0.89	2	1	0	0	1	0	0.11
	5 mg/ml	3680	895	24.32 ± 0.94	0	0	1	2	1	1	0.14
	10 mg/ml	3576	887	24.80 ± 1.12	0	0	1	1	0	0	0.05

$*P < .05$ in One Way ANOVA.

Discussion\

The *A. cepa* assay has been widely used for evaluation of cytotoxic and genotoxic activity of various compounds (9, 27, 16). *A. cepa* assay enabled the assessment of different genetic endpoints, which are mitotic index and chromosome aberration. Mitotic index was characterized by the total number of dividing cells in cell cycle. Mitotic index is used as an indicator of cell proliferation biomarkers which measures the proportion of cells in the mitotic phase of the cell cycle. Several types of chromosome aberrations were considered in the four phases of cell division (prophase, metaphase, anaphase and telophase) to evaluate chromosomal abnormalities, according to (28).

In the present study no significant difference was observed between the *J. phoenicea* treatment and the negative control, due to low cytotoxicity. However, a decrease could be observed in the mitotic index and in the frequency of chromosome abnormality values with the increase in the *J. phoenicea* Leaf Extracts concentration after 24 h exposure period, due to low cytotoxicity. Therefore, more studies are required to evaluate the appropriate dosage of this natural component during treatment. Chromosome aberrations were observed in very low frequency in all stages of mitosis. Table 1 shows the frequency of chromosome aberration in each treatment, and Figure 1 shows the types and frequency of each abnormality. Chromosome aberrations provided important information and may be considered an efficient test to investigate the genotoxic potential of the treatments analyzed (8). The chromosome aberrations observed at all concentrations of the treatment were chromosome aberrant cells include chromosome fragments, stickiness, polar deviation, Anaphase bridges, Lagging chromosome and Early condensation in prophase as compared with the negative control. These aberrations were due to the effect of the extract on the spindle formation and thus resulted in cell division disturbances. Mitotic irregularities such as irregular prophase and anaphase, fault polarization, alignment anaphase and bridges may be mainly the result of the above reasons. Disrupted equatorial plate may result from unequal distribution of chromosome and spindle dysfunction (Figure 1a), whereas Chromosome stickiness means loss of normal appearance, and it presents sticky surface, causing chromosome agglomeration. Disturbance during metaphase and anaphase arises because of the effect of the treatment on the spindle, which leads to failure of the spindle mechanism (Figure 1b) (26), reported that this stickiness may be interpreted as a result of depolymerisation of DNA, partial dissolution of nucleoproteins, breakage and exchanges of the basic folded fiber units of chromatids and the stripping of the protein covering of DNA in chromosomes, According to (15). The presence of chromosome fragments is an indication of chromosome breaks, and can be a consequence of anaphase/telophase bridges (Figure 1c) (34). The bridges noticed in the cells were probably formed by breakage and fusion of chromatids or subchromatids (Figure 1d) (33), According to (20) Lagging chromosome that were not organized to a specific stage of the mitotic division were also observed (Figure 1e). This abnormality may be caused by unequal distribution of chromosomes with paired chromatids in which resulted from nondisjunction of chromatids in anaphase.

There is an observed increase in the density of chromosomes during prophase, as the cell prepares to divide its contents. Meanwhile, the nuclear envelope is dissolving to allow the later events of mitosis to take place unhindered (Figure 1f). In another study, protective Role of *J. phoenicea* leaves extract against gamma-irradiation-induced oxidative stress. The results suggested that treatment with *J. phoenicea* leaves extract is possibly safe and can ameliorate gamma-irradiation-induced oxidative damage and tissue injury in rats (12). The leaves of *J. phoenicea* could serve as a potential source of therapeutic antioxidants, Furthermore; flavonoid contents of *J. phoenicea* inhibit the tumor growth by interfering with some phases of the cell cycle (32). In the same respect, *J. phoenicea* leaves were found to contain active components and due to these components; they show anti-proliferative activity against abroad range of human tumors (29) and it may have some effects against certain kinds of cancer (7).

The present study showed that *J. phoenicea* does not present chromotoxic and mitodepressive effects on meristematic cells of *A. cepa* for the tested concentrations. Therefore, more studies are required to evaluate the dosage effect on genotoxicity Using Spectrophotometric analysis of DNA concentration and Gel electrophoresis.

References\

1) **Aboul-Ela, M., El-Shaer, N and Abd El-Azim, T. 2005.** Chemical constituents and antihepatotoxic effect of the berries of *J. phoenicea* part II. *Natural Product Sciences*. 11: 240-247.
2) **Akkol, E., Güvenc, A., Yesilada, E. 2009.** A comparative study on the antinociceptive and anti-inflammatory activities of five *Juniperus* taxa *Journal of Ethnopharmacology*. 125:30-336.
3) **Ames, B. 1986.** Food constituents as a source of mutagens, carcinogens and anticarcinogens, in Genetic Toxicology of the Diet, I. Knudsen. 55–62, Alan R. Liss, New York, NY, USA.
4) **Amida, M., Yemitan, O and Adeyemi, O. 2007.** Toxicological assessment of the aqueous root extract of *Sanseviera liberica* Gerome and Labroy (Agavaceae). *Journal of Ethnopharmacology*. 113 (1):171-175.
5) **As¸kın, T., elik, C and Aslant ¨ urk, S. 2007.** Cytotoxic and genotoxic ¨ effects of *Lavandula stoechas* aqueous extracts, *Biologia*. 62. 3. 292–296.

6) **Bakare A., Mosuro A and Osibanjo, O. 2000.** Effect of simulated leachate on chromosomes and mitosis in roots of *Allium cepa* (L). *Journal of Environmental Biology*. 21(3):263–271.

7) **Bayazit, V. 2004.** Cytotoxic effects of some animal and vegetable extracts and some chemicals on liver and colon carcinoma and myosarcoma. *Saudi Medical Journal*. 25: 156-163.

8) **Carita, R and Marin-Morales, M. 2008.** Induction of chromosome aberrations in the *Allium cepa* test system caused by the exposure of seeds to industrial effluents contaminated with azo dyes. *Chemosphere*, *72*, 722–725.

9) **Del campo, D., Bracho, M., Marcano, L., Guinez, J and De la torre, C. 2005**. DNA injury induced by 5-aminouracil and caffeine in G2 checkpoints path of higher plant cells. Biocell. 29. 2. 169-176.

10) **Di Carlo, G., Mascolo, N., Izzo, A and Capasso, F. 1999.** Flavonoids: old and new aspects of a class of natural therapeutic drugs. *Life Sciences*. 65: 337-353.

11) **Effraim, K., Jacks, T and Sodipo, O. 2001**. Histopathological studies on the toxicity of *Ocimum gratissimum* leaves extract on some organs of rabbit. *African Journal of Biomedical Research*. 6:21-25.

12) **Eldessoky, S., Ismail, A., Ehab, I and Walaa, F. 2020.** Protective Role of *Juniperus phoenicea* L. Leaves Extract against Gamma-irradiation-induced Oxidative Stress. *Pakistan Journal of Biological Sciences*. 23(7):922-930.

13) **El-Sawi, S,. Motawae, H and Ali, A. 2007.** Chemical composition, cytotoxic activity and antimicrobial activity of essential oils of leaves and berries of *Juniperus phoenicea* L. grown in Egypt. *African Journal of Traditional, Complementary and Alternative Medicines*. 4: 417-426**.**

14) **Fernandes De Sa Ferreira, I and Ferr ´ ao Vargas, V. 1999.** Muta-˜ genicity of medicinal plant extracts in Salmonella/microsome assay, *Phytotherapy Research*.13. 5. 397–400.

15) **Fiskesjo, G. 1985.** The Allium test as a standard in environmental monitoring. *Hereditas.* 102:99-112.

16) **Frescura, V., Khun, A., Laughinghouse fourth, H., Paranhos, J and Tedesco, S. 2013.** Post-treatment with plant extracts us in Brazilian folk medicine caused a partial reversal of the antiproliferative effect of glyohosate

in the *Allium cepa* test. Biocell. 37. 2. 23-28.

17) **Grant, W. 1982.** Chromosome aberrations assay in Allium. A report of the U.S. Environmental protection Agency. *Gene-Tox* programme Mutation Research. 99:273-291.

18) **Harborne, J. 1993.** The Flavonoids: Advances in Research Since 1986. Chapman and Hall, London, UK. ISBN-13: 460-461.

19) **Higashimoto, M., Purintrapiban, J and Kataoka, K. 1993.** Mutagenicity and antimutagenicity of extracts of three spices and a medicinal plant in Thailand, Mutation Research. 303. 3. 135–142.

20) **Kabarity, A., El-Bayoumi, A and Habib, A. 1974.** Effect of morphine sulphate on mitosis of *Allium cepa* L. root tips. *Biol. Plantarum*, *16*, 275–282.

21) **Khora, S., Panda, K and Panda, B. (1997).** Genotoxicity of tetrodotoxin from puffer fish tested in root meristem cells of *Allium cepa*. *Mutagenesis*, 12. 4. 265-269.

22) **Konan, N., Bacchi, E., Lincopan, N., Varela, S and Varanda, E. 2007**. Acute, subacute toxicity and genotoxic effect of a hydroethanolic extract of the cashew (*Anacardium occidentale* L.). *Journal of Ethnopharmacology*. 110:30-38.

23) **Mosuro, A and Bakare, A. 1998**. Cytogenetic effects of lambdacyalothrin and paraquat on *Allium cepa*. *Journal of Engineering Science and* Technology. 5. (1):1055–1063.

24) **Moody, J., Ajaiyeoba, E., Adeboye, J and Ogundipo, O. 1999.** Standardization and utilization of herbal medicines, Proceedings of 1st International workshops on herbal medicinal products, Ibadan. 6-8.

25) **Nwafor, P., Egwu, G., Jahn, G and Deis, R. 2007.** Effects of methanolic extract of *Asparagus pubescens* root on sexual behavior and pituitary hormone secretion on Wistar rats during pregnancy and lactation. Journal of Ethnopharmacology, 113(3):492-497.

26) **Leme, D and Marin-morales, M. 2009.** *Allium cepa* test in enverionmental monitoring: a review on its application. Mutation Research, 682(1): 71-81.

27) **Liu, D., Jiang, W., Meng, Q., Zou, J., Gu, J. and Zeng, M. 2009.** Cytogenetical and ultrastructural effects of copper on root meristem cells of *Allium sativum* L. Biocell.33. 1. 25-32.

28) **Rank, J. and Nielsen, M. 1997.** *Allium cepa* anaphase-telophase root

tip chromosome aberration assay on N-methyl-N-nitrosourea, maleic hydrazide, sodium azide, and ethyl methanesulfonate. Mutation Research - Genetic Toxicology and Environmental Mutagenesis, 390: 121–127.

29) **Rizk, M., Abdallah, M., Sharara, H., Ali, S; Ibrahim, N and Mustafa, M. 2007.** Efficiency of *Cupressus sempiverens* L. and *Juniperus phoenicea* against carbon tetrachloride hepatotoxicity in Rats. Trends in Medical Research, 2 (2): 83-94.

30) **Ruffa, M., Ferraro, G., Wagner, M., Calcagno, M., Campos, R and Cavallaro, L. 2002.** Cytotoxic effect of Argentine medicinal plant extracts on human hepatocellular carcinoma cell line. Journal of Ethnopharmacology, 79:335–339.

31) **Plewa, M and Wagner, E. 1993.** Activation of promutagens by green plants. Annual Review of Genetics, 27: 93–113.

32) **Salucci, M., Stivala, L., Maiani, G., Bugianesi, R and Vannini, V. 2002.** Flavonoids uptake and their effect on cell cycle of human colon adenocarcinoma cells (CaCo2). British Journal of Cancer, 86(10): 1645–1651.

33) **Shehab, A and Adam, Z. 1983.** Cytological effects of medicinal plants in Qatar III. Mitotic effect of water extract of *Anastatica hierochuntico* L. on *Allium cepa.* Cytologia, 48: 343–348.

34) **Sharma, A and Sen, S. 2002.** Chromosome Botany, Science, Enfield, NH, USA, 2002.

35) **Teixeira, R., Camparoto, M., Mantovani, M and Vicentini, V. 2003.** Assessment of two medicinal plants, *Psidium guajava* L. and *Achillea millifolium* L., in invitro and in vivo assays. Genetics and Molecular Biology, 26(4):551-555.

36) **Uphof, J. 1968.** Dictionary of Economic Plants. 2nd Edn. Verlag Von Journal. Cramer, Germany. 290.

الملخص العربى/

تقييم السمية الخلوية والسمية الجينية لمستخلص أوراق العرعر الفينيقي (*Juniperus phoenicea*) باستخدام اختبار البصل (*Allium cepa*).

مبروكة عبدالله جبريل عبدالرحيم

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استخدم العرعر الفينيقي (*Juniperus phoenicea* L.) لسنوات في الطب الشعبي كمضاد للالتهابات ومضاد للأكسدة. و في هذه الدراسة، تم فحص التأثيرات السمية الخلوية والوراثية لمستخلص أوراق العرعر الفينيقي و تتضمن المعاملة المباشرة للقمم النامية لنبات البصل (*Allium cepa* L.) وذلك لمعرفة تأثير هذا المواد الكيميائية الموجودة في هذا المستخلص علي الخلايا، ثم خضعت للتحليل المجهري باستخدام صبغة الأسيتوكارمين، تم استخدام الماء المقطر للمقارنة (الضابط). وأظهرت النتائج، في ظل ظروف الاختبار، أن معدل الانقسام الخلوي (Mitotic index) لجذور البصل الخاضعة للمعاملة بمستخلص أوراق العرعر الفينيقي لم يختلف اختلافًا كبيرًا عن الضابط مما يشير إلى أن العرعر الفينيقي ليس سامًا للخلايا. كما لوحظ انخفاض حدوث انحرافات الكروموسومات في الخلايا المعاملة بمستخلص أوراق العرعر الفينيقي، مما يشير إلى أن العرعر الفينيقي ليس له تأثير سام علي المادة الوراثية أيضًا. مما يتطلب المزيد من الدراسات لتقييم تأثير الجرعة على السمية الجينية باستخدام تقنية المطياف الضوئي (Spectrophotometer) و تقنية الهجرة الكهربائية (Gel electrophoresis).

الكلمات المفتاحية: السمية الخلوية ، السمية الجينية ، العرعر الفينيقي ، جذور البصل.