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CYTOLOGICAL ANALYSIS OF THE ANTI-MUTAGENIC EFFECT OF *Tamarix nilotica* PLANT IN MICE BONE MARROW

[152]

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ABSTRACT

The usages of medicinal plants as therapeutic agents have been practiced in a large scale of applications that make studies of their mutagenicity and/or anti-mutagenic /anti-carcinogenic effects very essential. The current investigation is focused on the anti-mutagenic effects of the *Tamarix nilotica* (Ehrenb) crude extract using chromosomal aberrations analysis in mice bone marrow. In fact, a single plant may have diversity of phytochemicals ranging from bitter compounds that stimulate digestive system, phenolic compounds for antioxidants and many other pharmacological properties, antibacterial, and antifungal, tannins that work as natural antibiotics, diuretic substances and alkaloids, etc. *Tamarix* is represented in Egypt with two indigenous species which are *Tamarix aphylla* (L.) (H.Karst) and *T. nilotica* (Ehrenb.) Bunge (*T. nilotica* (Ehrenb.). In addition, it was used in Egyptian traditional medicine as an antiseptic agent. Extracts of *Tamarix* species have been widely used in traditional medicine in Asia and Africa mainly for their antiseptic, astringent, diaphoretic and diuretic properties. The current investigation is focused on the anti-mutagenic effects of the *Tamarix nilotica* crude extract using chromosomal aberrations analysis in mice bone marrow. Mitomycin C (MMC) was administered to mice as a positive control alone before and after treatment with 5 or 0.5 mg/kg b.wt *Tamarix* crude extract as acute (24 and 48 h) and sub-acute (15 consecutive days) doses respectively. Results indicated that the Mitomycin C (MMC) exposure induced statistically significant increase in chromosomal aberrations compared to the control, however *T. nilotica* revealed slight insignificant effect on aberrant mitosis rate. Chromosomal aberration domain structural and numerical aberrations.

The frequency of chromosomal aberrations (CA) and mitotic index (MI) decreased with increasing the dose and time of *T. nilotica* treatment, especially pre-treatment (plant + MMC). This effect was found to be dose-dependent. In conclusion, the results showed that *T. nilotica* could be considered as a good anti-mutagenic agent.

Keywords: Medicinal plants, Anti-mutagenic, *Tamarix nilotica,* Chromosomal aberrations

INTRODUCTION

Use of anti-mutagens and anti-carcinogens in everyday life has been suggested to be the most effective procedure for preventing human cancer and genetic diseases (**Ferguson, 1994**). Recently, there has been a global surge in the popularity of herbal/ traditional medicine, and currently there is enormous interest in developing new pharmaceutical products from such resources (**Fahmy et al 2007**).

Medicinal Plants are still the mainstay of about 70-80% of the world population, largely in developing countries, for primary healthcare needs because of better cultural acceptability, better compatibility with human body and lesser side effects (**Kamboj, 2000**). In fact, a single plant may have diversity of phytochemicals ranging from bitter compounds that stimulate digestive system, phenolic compounds for antioxidants and many other pharmacological properties, antibacterial, and antifungal, tannins that work as natural antibiotics,

diuretic substances and alkaloids, etc. (**Miguel, 2010**). *Tamarix* is represented in Egypt with two indigenous species which are *Tamarix aphylla* (L.) (H.Karst) and *T. nilotica* (Ehrenb.) Bunge (**Boulos, 1999**). *T. nilotica* (Ehrenb.) Bunge has its root deep in the Egyptian history where it was mentioned in ancient papyri in pharaonic times to heal from fever, relieve headache, to draw out inflammation and as an aphrodisiac. In addition, it was used in Egyptian traditional medicine as an antiseptic agent (**Abouzid and Sleem, 2011**). In Egypt, different parts of *Tamarix* are used; the leaves and young branches are cooked for oedema of spleen and mixed with ginger for uterus infections, while the bark, when boiled in water with vinegar is used as lotion against lice (**Boulos, 1983**). Extracts of *Tamarix* species have been widely used in traditional medicine in Asia and Africa mainly for their antiseptic, astringent, diaphoretic and diuretic properties (**Bulos, 1983**). Moreover, antioxidant and hepatoprotective activities were evaluated for total flower extract of *Tamarix* (**Abouzid et al 2008 and Abouzid & Sleem, 2011**). In addition, **Orabi et al (2010**) stated that a high dietary intake of flavonoids could be associated with low cancer prevalence in humans, the cytotoxicity of some phytochemicals from the leaves of *T. nilotica* to human promyelocytic leukemia and human squamous carcinoma cell lines have been reported. Therefore, *T. nilotica* appeared promising as a natural source for new drugs (**Barakat et al 1987**). The short term mutagenicity assays were proved to be good tools to assess the mutagenicity and predicted carcinogenicity of single compounds as well as mixed compounds either in direct applications or under environmental exposure. Mice chromosomal aberrations assay was used for long time as a well valid and sensitive assay to evaluate the clastogenic effect of mutagenic agents (**Tice et al 1994**). Therefore, the present study aimed to assess the genotoxic properties of Ethyl acetate crude extract of *Tamarix nilotica* through the incidence of chromosomal aberrations in mice.

MATERIALS AND METHODS

Plant collection and extract preparation

Tamarix nilotica (Ehrenb) plant was collected from Wadi Gharandal, South Sinai, Egypt in March 2015 by Desert Research Centre (DRC).

Collection and preparation of plant extract

The methods and protocols followed by **Bakr et al (2013)** were used with modification as the following:

Fresh leaves were washed and air dried at room temperature and were milled into powder. Extract was obtained by shaking 250 g plant powder in 500 ml of Ethyl acetate .It was left for 24 h at 37°C for 3 days, filtered by using filter paper, evaporated under vacuum and the yield was (2.43 g) of (1000 g) powder. After drying under vacuum, the extracts were then dried and finally placed in glass vials and re-suspended in corn oil until using.

Experimental animals

Sixty five albino male mice, weighing 25g ± 2, were obtained from the animal house of the National Research Centre, Dokki, Cairo, Egypt. and kept for one week for acclimatization prior to starting the study.

Dosage and treatment

Two doses were used in this study

1. Low dose 0.5 mg/kg.
2. High dose 5 mg/kg.

The two doses of *Tamarix* were orally given acutely (24 & 48 h), sub-acutely (15 consecutive days), pre-treatment (plant extract + MMC) and post treatment (MMC + plant extract).

Preparation of somatic cell chromosomes (bone marrow)

Mice were sacrificed 24 h after administration of the last treatment for chromosome aberrations analysis. Bone marrow metaphases were prepared according to the recommendations of **Yosida et al** (**1965**) with slight modifications. Experimental animals were injected interperitonealy (i.p.) with colchicines (0.4 mg/kg) 2 h before sacrifying.

Femoral bone marrow was flushed with physiological saline solution (NaCl 0.9%). The cells were centrifuged at 1000 rpm for 10 minutes and the supernatant was discarded. The pellet was redispersed in a hypotonic solution of (KCl 0.56 gm/100 ml of distilled water) and incubated at 40°C for 30 minutes to permit osmotic swelling of cells suspensions. The cells were centrifuged at 1000 rpm for

10 min, fixed in methanol: glacial acetic acid (3:1 v/v). Centrifugation and fixation were repeated 3 times at least. The cells were re-suspended in a little volume of fixative, placed onto chilled slides, flame-dried and stained in 10% phosphate buffered Giemsa (pH: 6.8) for 30 min and left to dry again. At least 50 good metaphase spreads for each animal were studied microscopically for scoring the different types of structural aberrations such as deletions, breaks, translocation, inversion, centric fusion, fragmentation, end to end association, gaps, sub-chromatid exchange, centromeric attenuations and numerical changes with selection based on uniform staining quality, lack of overlapping chromosomes and numerical abberations such as polyploidy, polysomy, monosomy and nullisomy at a magnification of 100x.

Mitotic index (MI)

The mitotic index (MI): (number of dividing cells in 1000 cells) was used to determine the mitotic activity of bone marrow cells.

Statistical analysis

All data were expressed as means ± standard error (SE). The obtained data were subjected to one way analysis of variance (ANOVA). According to **Snedecor and Cochran,** (**1980**) using SPSS 18.0 software. Least significant differences LSD were used to compare between mean of treatments at probability of 5%.

RESULTS

Chromosomal aberrations and mitotic activity

Table (1) shows chromosomal aberrations and mitotic activity in somatic cells after acute (24 and 48 h) and sub-acute (15 consecutive days) treatments with *Tamarix nilotica (T. nilotica)* tested extract, and its anti-mutagenic activity against the clastogenic effects of mitomycin C (MMC) using *in vivo* clastogenicity assay of mouse bone marrow cells. Two doses (low 0.5 mg, and high 5 mg) of *T. nilotica* extract were tested alone or in combination with MMC as pre and post treatments compared to the negative and positive MMC controls.

Chromosomal aberrations were mainly of the chromatid type and include gaps, deletions and centrometric attenuations **(Fig. 1b, c and d)**. *T. nilotica* treatments showed slight non-significant increase in chromosomal aberration frequency. On the other hand, MMC induced highly significant increase of all types of chromosomal aberrations (18.6 ± 1.6913) after 24 h. of administration. It also induced marked clastogenic effects, as it reduced mitotic activity **(Table 1)**.

In pre-treatment, administration of *T. nilotica* for long time (15 consecutive days) before MMC caused a marked decrease in the aberrant cells. The results showed a significant decrease in chromosomal aberrations from (18.6 ± 1.6913) to (12 ± 3.1143) in the group receiving low dose (0.5 mg/kg). While in the high dose (5 mg/kg) a highly significant decrease from (18.6 ± 1.6913) to (9.6 ± 2.9766) in chromosomal aberration was observed.
In post treatment, administration of low dose (0.5 mg/kg) *T. nilotica* for 15 consecutive days after MMC treatment showed slight insignificant differences in the frequency of chromosomal aberrations caused by MMC. While the high dose (5 mg/kg) of *T. nilotica* decreased the percentage of chromosomal aberrations from (18.6 ± 1.6913) to (14.4 ± 1.2889). This showed a dose dependent effect of *Tamarix.*

Effect on mitotic activity

The mitotic index (MI) was used to assess the rate of cell division. The slides prepared for the assessment of chromosomal aberrations were also used for calculating the mitotic index. **Table (1)** illustrated the percentage of mitotic index in which *T. nilotica* with different doses revealed mitotic activity to be similar to negative control.

Moreover, our study demonstrated that the decrease in MI was lower in MMC than the negative control and *Tamarix* treatments. The mitotic index after treatment with MMC was 55%, while in the negative control it was 80%. Mitotic indices are summarized in **Table (1)**.

As a result, all samples showed lower percentage of aberrations in acute and sub-acute treatments compared to MMC effect. This effect was found to be dose dependent and showed the antioxidant activity of *T. nilotica*. Also both pre and post-treatments of *T. nilotica* extract were capable to reduce the frequency of chromosomal aberrations induced by MMC.

Table 1. Statistical analysis of chromosomal aberrations in bone marrow cells of male mice treated with MMC and/or *T. nilotica* extract

Treatments	Structural aberrations			Total structural aberrations	Numerical aberration		Total numerical aberrations	Mitotic activity	
	Deletion	Gap	Centromeric attenuation		> 2n	< 2n		No. of examed cells	MI
Control	0.4 ± 0.2450	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.2450	0.4 ± 0.3998	0.2 ± 0.1999	0.6 ± 0.3998	10000	80
MMC	11.2 ± 1.8277	1.6 ± 0.3998	5.8 ± 1.3563	*18.6 ± 1.6913	3.2 ± 0.8604	13.8 ± 0.8604	*17 ± 1.2249	10000	55
Oil 15 Days	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.8 ± 0.8000	0.4 ± 0.3998	1.2 ± 0.8000	10000	70
T. nilotica (0.5 mg/kg) 24 h	0.4 ± 0.2450	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.2450	0.6 ± 0.6001	0.6 ± 0.6001	1.2 ± 0.7347	10000	72
T. nilotica (0.5 mg/kg) 48 h	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1 ± 0.6323	0.0 ± 0.0	1 ± 0.6323	10000	73.5
T. nilotica (0.5 mg/kg) 15 d	0.2 ± 0.1999	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1999	0.4 ± 0.3998	0.4 ± 0.3998	0.8 ± 0.4896	10000	74.2
Pre-treatment (0.5 mg/kg) 15 d	9.6 ± 2.6381	2.4 ± 0.8125	0.0 ± 0.0	*12 ± 3.1143	6.6 ± 1.3997	4.4 ± 1.5035	*11 ± 2.1680	10000	76
Post-treatment (0.5 mg/kg) 15 d	14.6 ± 0.9275	2.4 ± 1.4351	0.0 ± 0.0	*17 ± 1.5165	3.6 ± 1.8054	10.4 ± 1.5035	*14 ± 3.1465	10000	70
T. nilotica (5 mg/kg) 24 h	0.2 ± 0.1999	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1999	1 ± 0.6323	0.0 ± 0.0	1 ± 0.6323	10000	73.5
T. nilotica (5 mg/kg) 48 h	0.2 ± 0.1999	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1999	0.4 ± 0.3998	0.4 ± 0.3998	0.8 ± 0.4896	10000	75
T. nilotica (5 mg/kg) 15 d	0.2 ± 0.1999	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1999	0.0 ± 0.0	0.2 ± 0.1999	0.2 ± 0.1999	10000	75.5
Pre-treatment (5 mg/kg) 15 d	7.6 ± 3.2342	2 ± 0.8367	0.0 ± 0.0	*9.6 ± 2.9766	3.6 ± 0.7481	4.4 ± 1.2884	*8 ± 1.6430	10000	78
Post-treatment (5 mg/kg) 15 d	14.2 ± 1.4284	0.2 ± 0.1999	0.0 ± 0.0	*14.4 ± 1.2889	2 ± 0.9485	10.6 ± 2.3791	*12.6 ± 2.8035	10000	75

* The mean difference is significant at the .05 level.

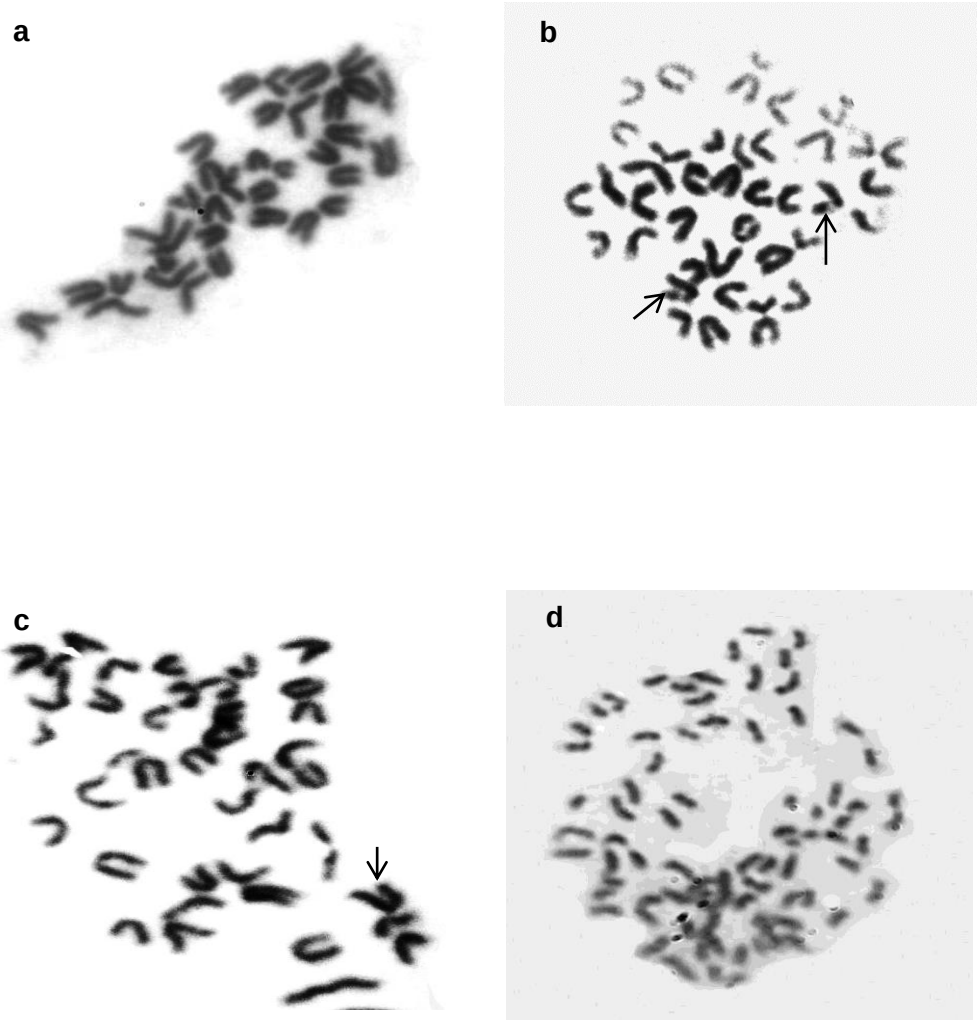

Fig. 1. Metaphase spreads from bone marrow of mice:

a) Normal metaphase spread b) chromatid gap

c) chromatid deletion d) centromeric attenuation.

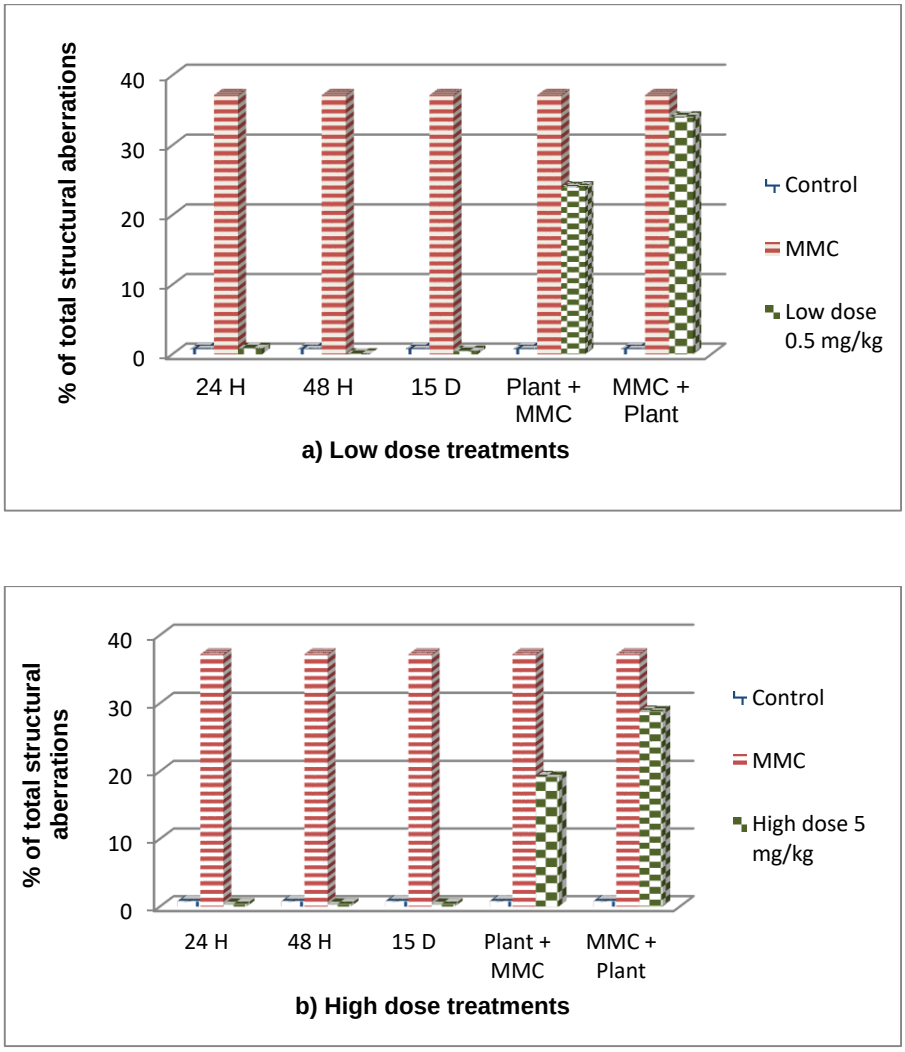

Fig. 2. (a and b). Frequencies of different cells with chromosomal aberrations induced by MMC and/or *Tamarix nilotica* extract in mice bone marrow cells

DISCUSSION

Medicinal plants are the primary agents of health care known to mankind. Natural products are important sources of anti-mutagenic and anti-cancer leading molecules and this is mainly due to the high degree of diversity and novelty. The increased interest in the measurement and use of plant antioxidants for scientific research, as well as industrial purposes, are mainly due to their strong biological activity, exceeding those of many synthetic antioxidants which have possible activity as promoters of carcinogenesis, in addition to safe and effective use with fewer side effects (**Suhaj, 2006 and Tadhani et al 2007).**

The results showed that MMC caused a significant increase in chromosome aberrations in bone marrow cells. MMC and colchicine are known as cytotoxic agents and used in some medicinal treatments. MMC used in treatment of gastric, pancreatic, oesophageal carcinomas and bladder cancer. The cytotoxicity of MMC act through the formation of DNA adducts and interstand cross-link (**Paz et al 2004 and Lee et al 2005**). It induces chromosome damage during the S phase (**Alder, 1984**). The DNA damage and the adverse events such as skin toxicity and cardiovascular may be related to the formation of reactive oxygen species (**Gutierrz, 2000**). Although MMC is an effective anticancer drug, its clinical use is restricted for its toxicity.

The results of this study showed a significant increase in chromosome aberrations in somatic cells due to exposure to MMC as well as the chemoprotection effect of *T. nilotica* extract against this damage.

However, crude extract of *T. nilotica* was found to induce substantial anti-mutagenic effect. Out of all types of structural aberrations; gaps, deletions and centromeric attenuations were the predominant forms of CA observed. In addition, some numerical aberrations were observed. Marked increases in the percentage of mitotic index values were noted in *Tamarix* groups as compared to the positive controls. This could be due to a slower progression of cells from S (DNA synthesis) phase to M (mitosis) phase of the cell cycle as a result of MMC exposure (**Patlolla et al *2010***). Although it is most likely that this impairment in cell cycle progression is associated with MMC toxicity.

These results were in partial agreement with those of **El-Din et al (2018)** who reported that *Zygophyllum album* extract decreased the chromosomal aberration in mice bone marrow.

Abou-Zid and Sleem (2011) reported that ethanolic extract of *T. nilotica* is rich in many phenolic and flvonoid constituents. They also suggested the high occurance of antioxidant activity of ethanolic extract of *T. nilotica.*

Also, the pre and post treatment analysis revealed that *T. nilotica* crude extract may contain some compounds can act as dis-antimutagens and other compounds that can act as bio-antimutagens. The results suggest that *Tamarix* does not possess a hazard on chromosomal integrity when used as herbal medicine, but still need more studies on the molecular level to assay its effects on DNA, and to establish the mechanism of action of these compounds in *T. nilotica* extract as anti-mutagen.

CONCLUSION

In conclusion, the result indicated that *T. nilotica* exhibited substantial anti-mutagenic activity in mice as indicated by its beneficial action of markedly reducing the levels of chromosomal aberrations.

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التحليل الوراثي السيتولوجي للتأثير المضاد للطفور لنبات الأثل النيلي في نخاع عظام فئران المعمل الصغيرة

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الموجـــــــز

للنباتات الطبية أهمية كبيرة في العديد من المجالات أهمها الصناعات الدوائية و علاج الأمراض المزمنة، وكانت هذه الدراسة على أحد النباتات وهو المعروف بالأثل النيلي *Tamarix nilotica* .

تم تقييم النشاط المضاد للطفور لمستخلص الإيثيل اسيتات لنبات *Tamarix nilotica* في الفئران للكشف عن التغيرات الكروموسومية الناتجة في خلايا نخاع العظام بإستخدام تركيزين هما: تركيز منخفض 0.5 مل جم/كجم من وزن الجسم. تركيز عالي 5 مل جم / كجم من وزن الجسم.

وأجريت المعاملات علي النحو التالي:

معاملة المجموعة الضابطة السالب. معاملة الكنترول الموجب بإستخدام ماده الميتوميسين سي Mitomycine C لمده 24 ساعة. معاملة كل من التركيزات المستخدمة للدراسة لمده 24 و 48 ساعة و15 يوم. معاملة كل من التركيزات المستخدمة للدراسة + الميتوميسين سي لمده 15 يوم. معاملة الميتوميسين سي+ كل من التركيزات المستخدمة للدراسة لمده 15 يوم.

دلت النتائج على أن المستخلص أعطى نسبه أقل من التغيرات الكروموسومية في خلايا نخاع العظام عنها في معاملة الميتوميسين سي حيث كلما زاد تركيز النبات ومدة المعاملة أصبح معدل الطفور أقل.

أوضحت النتائج أن استخدام مستخلص النبات في المعاملات الحادة وشبه الحادة لم تحدث زيادة معنوية في نسبة التغيرات الكروموسومية بالمقارنة مع المجموعة الضابطة السالب.

كما أوضحت النتائج أن المعالجة الأولية والمعالجة النهائية للحيوانات التي تم حقنها بالميتوميسين سي مع مستخلص النبات أظهرت انخفاض معنوي في نسبه التغيرات الكروموسومية الناتجة من استخدام الميتوميسين سي وخاصه في المعالجة الأولية.

وبذلك يتبين من النتائج أن *Tamarix nilotica* يعد مصدر طبيعي جيد للعوامل المضادة للطفور أو استحداث التغيرات الكروموسومية في فئران التجارب.

الكلمات الدالة: النباتات الطبية، الأثل النيلي، التغيرات الكروموسومية، مضاد للطفور

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