Evaluating the Genotoxicity Enhancement of the Antileukemic Drug 6-Mercaptopurin When Combined With Iraqi *Nerium oleander* and *Narcissus tazetta* Extracts *in vivo*

تقييم تعزيز السمية الجينية للعقار المضاد لسرطان الدم 6- مركابتوبيورين عند اعطائه مع مستخلص نباتي الدفلى والنرجس العراقيين داخل الجسم الحي

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Abstract

The present study was aimed to evaluate the genotoxicity of the aqueous extracts of *Nerium oleander* leaves and *Narcissus tazetta* bulbs each alone and together with the antileukemic drug 6-Mercaptopurin (6MP) in order to investigate the extracts ability to elevate the chemotherapeutic drug genotoxicity which may influence its treatment of cancer. The cytotoxicity test shows that the LD50 of the aqueous extract of *Narcissus tazetta* was 752.083 mg/kg and for *Nerium oleander* was 922.023 mg/kg. On the basis of the achieved LD50 values, the doses 92, 46 ,23 mg/kg of *Nerium oleander* and the doses 75, 37,18 mg/kg of *Narcissus tazetta* extracts were chosen, depending on chromosome aberrations, Micronuclei and Mitotic index as a powerful cytogenetic assays in bone marrow cells of Swiss albino male mice. The result indicated that *Nerium oleander* extract alone at the dose 92mg/kg induced significant effect on centromere break and ring chromosome comparing with the negative control (untreated mice) and significantly increased the mean values of chromatid gap and ring chromosome when compared with the positive control (6MP). While only the dose 46 mg/kg and 23 mg/kg of *N. oleander* aqueous extracts significantly decreased mitotic index and when combined with 6MP it can enhance its antimitotic activity but not significantly. Moreover the extracts alone and when combined with 6MP did not significantly change the total number of red blood micro nucleated cells. For *Narcissus tazetta* extract, the three experimental doses alone lead to significant increase in chromosomal aberrations like: chromatid break with fragment, chromatid break without fragment, chromatid gap, centromeric break, ring chromosome and dicentric chromosome. While only the dose 75mg/kg had induced significant structural chromosomal abnormalities such as chromatid break with fragment, chromatid break without fragment centromeric break and ring chromosome, when combined with 6MP. The three doses of *N. tazetta* extract alone, had led to significant reduction in mitotic index compared with untreated control and also its combination with 6-MP significantly decreased the percentage of mitotic index. Moreover, only the doses 75 mg/kg and 37 mg/kg of *N. tazetta* extracts, when had given alone caused significant increase of the total micronucleated cells. While only the dose 75mg/kg of *N. tazetta* had induced significant frequency of the total micronucleated cell when combined with 6MP. In the present report, we attempted to establish that *N.tazetta* and *Nerium oleander* aquatic extracts enhance the genotoxicity and bioactivity induced by the antileukemic drug 6MP , thus preventing the development of cellular drug resistance which is a major problem that can face cancer patients using this drug .The current study serve the purpose of which is to search for local plants that may contribute to the establishment of novel supportive complementary and alternative medicine (CAM) during the chemotherapy of cancer in Iraq , Further studies are merited to explore this possibility.

Key words: Mitotic index, Micronuclei, Chromosomal anomalies, *Nerium oleander* leaves, *Narcissus tazetta* bulbs

الملخص

هدفت الدراسة الحالية إلى تقييم السمية الجينية لكلا المستخلصين المائيين لأوراق نبات الدفلى وابصال نبات النرجس كلا على حده ومع العقار المضاد لسرطان الدم 6 مركابتوبيورين من أجل الكشف عن قدرة المستخلصات النباتية على رفع السمية الوراثية لهذا العقار مما قد يؤثر على استخدامه في العلاج الكيميائي للسرطان . واظهر اختبار السمية الخلوية أن الجرعة الوسطية السامه للمستخلص المائي

للنرجس كانت 752.083 ملغم / كغم بينما لمستخلص الدفلى فكانت922.023 ملغم / كغم في خلايا نخاع عظم الفئران البيضاء واعتمادا على هذه النتيجة تم اختيار الجرعات92، 46، 23 ملغم / كغم لمستخلص اوراق الدفلى والجرعات 75، 37، 18 ملغم / كغم لمستخلص ابصال النرجس وبالاعتماد على اختبار التشوهات الكروموسوميه واختبار النويات الصغيرة فضلا عن اختبار معامل الانقسام الخلوي. واشارت النتائج أن مستخلص الدفلى لوحده و بالجرعه92 ملغم / كغم استحث زياده معنويه في قيم الكسور السنتروميريه والكروموسومات الحلقية مقارنة بالسيطرة السالبة أي بالفئران غير المعاملة و حينما اعطي المستخلص مع العقار 6 مركابتوبيورين وجد ان الجرعة 92 ملغم / كغم ايضا استحثت معنويا قيم الكسور السنتروميريه والكروموسومات الحلقية عند مقارنتها مع السيطرة الموجبة, في حين ان الجرع 46 ملغم / كغم و23 ملغم / كغم من مستخلص الدفلى قد خفضت بشكل ملحوظ من مؤشر الانقسام الخلوي. وعند دمج جرع المستخلصات الثلاث مع 6 مركابتوبيورين فقد وجد انها عززت قدرة العقار المثبط للخلايا لكن ليس بشكل معنوي. وبالإضافة الى ذلك وجد ان مستخلصات الدفلى الثلاث لوحدها او حين اعطائها مع العقار الكيمياوي لم تؤثر معنويا على العدد الاجمالي للنويات الصغيرة في خلايا الدم الحمر لنخاع العظم. اما بالنسبة لمستخلص ابصال النرجس فقد وجد ان المستخلصات التجريبية الثلاث لوحدها ادت الى زياده معنويه في عدد من التشوهات الكروموسوميه مثل :الكسور الكروماتيديه مع الشظايا, الكسور الكروماتيديه بدون الشظايا ,الفجوات الكروماتيديه والكسور السنتروميريه ,الكروموسومات الحلقية والكروموسومات ثنائية السنترومير. وعند اعطاء المستخلص مع العقار 6 مركابتوبيورين وجد ان الجرعة 75 ملغم / كغم من مستخلص ابصال النرجس تسببت في استحثاث تشوهات تركيبيه كروموسوميه معنويه مثل الكسورالكروماتيديه مع الشظايا، كسوركروماتيديه بدون شظايا، كسور سنتروميريه و كروموسومات حلقيه. وعلاوة على ذلك فان المستخلصات الثلاث لوحدها ادت الى انخفاض معنوي في معامل الانقسام الخلوي مقارنه بالسيطرة السالبة مثلما ادى اتحاد هذه المستخلصات مع العقار الكيمياوي الى خفض معنوي لنسبة الخلايا المنقسمة لنخاع العظم مقارنه مع السيطرة الموجبة. ووجد ان الجرعات 75 ملغم / كغم و37 ملغم / كغم من مستخلص النرجس لوحده تسببت في زياده معنويه في اجمالي النويات الصغيرة , بينما عند اعطاء الجرع الثلاث للمستخلص مع العقار وجد ان الجرعة 75 ملغم / كغم فقط هي التي استحثت زياده معنويه في المعدل الاجمالي للنويات الصغيرة مقارنه بالسيطرة الموجبة. في هذه الدراسة حاولنا ان نثبت ان مستخلصي نبات الدفلى والنرجس المائيان قد عززا السمية الوراثية والفعالية البيولوجية للعقار التقليدي المضاد لسرطان الدم 6 مركابتوبيورين وكنتيجة لذلك يمكن لهذه المستخلصات ان تمنع من تطور الخلايا المقاومة للعقار التي تعتبر مشكله اساسيه لدى استخدامه من قبل مرضى السرطان هذا فضلا عن ان استخدام مستخلصي هذين النباتين كلا على حده مع العقار 6 مركابتوبيورين قد يساهم في الكشف عن نباتات محليه تستخدم للمرة الاولى في إنشاء ودعم الطب التكميلي والطب البديل خلال العلاج الكيميائي للسرطان في العراق, دراسات اخرى ضرورية للكشف عن امكانية ذلك.

الكلمات الدالة: معامل الانقسام الخلوي , التشوهات الكر وموسوميه , النويات الصغيرة ,اوراق نبات الدفلى ,ابصال نبات النرجس

Introduction

Treating of cancer involves many steps, which include radiotherapy, surgery and chemotherapy. Development of chemoresistance is a major factor in the failure of many forms of chemotherapy, so increasing the drug effect or decreasing the resistance to the drug by different mechanisms is one goal of the scientists [1].

In cancer, chemotherapy represents the backbone of treatment for many cancers at different stages of the disease; therefore, enhancing the efficacy of chemotherapeutic drugs is need to avoid the therapeutic failure and eventually death [2]. Many conventional chemotherapeutic drugs are known to be involved in DNA damage, thus ultimately leading to apoptosis of leukemic cells. However, they fail to completely eliminate leukemia stem cells due to their higher DNA repair ability of cancer stem cells than that of cancer cells, which becomes the origin of drug resistance and leukemia repetition [3]. The conventional chemotherapeutic drug, 6-Mercaptopurine (6-MP) is a Purine antimetabolite which used for the treatment of acute leukemia; it is a purine analog that inhibits nucleic acid synthesis. However, resistant tumor cells develop rapidly, probably because of altered specificity or lack of phosphoribosyl transferases, which leads to inactivate thio-IMP (the active inhibitor). Other mechanisms may include altered cell permeability and an increased rate of destruction of 6-mercaptopurine [4,5].

Attempts to enhance chemotherapeutic drugs mainly involve the use of combination drug therapy using different classes of drugs with minimally overlapping toxicities to allow maximal dosages and with narrowest cycle intervals, necessary for bone marrow recovery [1]. Another growing field in health care and particularly among cancer patients, is using the complementary and alternative medicines (CAM) [6] knowing that herbal medicinal products (HMPs) belong to a main part of complementary and alternative medicine (CAM). Herb-drug interaction is actually a new field of research that has taken in consideration by researchers for its important value in health assurance [7]. Complementary and alternative therapies are becoming more common ways to achieve an improved quality of cancer patient's life. The potential risks of concurrent administration are serious and must be addressed because the synergistic potential between herbal medicines and drugs can be therapeutically advantageous or disadvantageous and requires physicians to be aware of the potential risks and benefits that might arise, clearly stressing the need for extensive work to be done in this new important and interesting area. However, comprehensive evidence for the risks and benefits of combining anticancer drugs with traditional herbs is rare [8,9].

To the best of our knowledge ,there was no previous investigation to find the correlation of 6-Mercaptopurine with the folk herb medicinal use, therefore, this study was designed to evaluate the probable enhancement of the genotoxicity effects of *Nerium oleander* and *Narcissus tazetta* extracts when given together with 6-mercaptopurine.

For *Nerium oleander* L. leaves, it is quite toxic in its raw form, though not so when prepared in some extracts, it has been observed that herbal tea made from oleander leaves or topical poultices made from crushed oleander leaves and flour can be used to heal wounds and skin cancers [10]. Moreover, Oleander has been used in the treatment of cardiac illness, asthma, diabetes mellitus, corns, scabies, and epilepsy [11], also many scientist observed that *Nerium oleander* contain different bioactive substance that can inhibit cancer cell [12] [13].

While *Narcissus tazetta* L. with the common name of "Narjes"have many medicinal uses due to containing many active constituents as secondary metabolites as alkaloids, the plant bulb is recommended for treatment of wounds and inflammation which has reported in traditional medicine of Turkey ,Persian , China, and Jordan, [14]. The bulb extract represents an interesting active ingredient and many researchers indicate that, narcissus could be used as anti-viral, anti-fungal, anti-tumor and many other uses [15,16].

Therefore, the aim of the present study was to investigate whether, Nerium *oleander* and *Narcissus tazetta* extracts, each alone or in combination with the chemotherapeutic agents 6-Mercaptopurine would elevate the inhibition of cell proliferation and strengthen 6-MP clastogenicity using bone marrow cells of Swiss albino mice as a model. Moreover, our study may assist in providing a theoretical basis to facilitate the development of a novel combinatorial approach to treat patients with Leukemia. Further studies are needed to explore this possibility.

Materials and Methods

Experimental Animals

A total number of two hundred and six mice (206) apparently healthy adult male Swiss albino mice (*Mus musculus*), Strain Balb/c of about (10-12) weeks age and weighting between (25-30) grams, were used for this study. These animals were obtained from the animal house of Science College, University of Sulaimani, Iraq. The animals were housed in plastic cages, five per cage, and maintained on standard laboratory diet and water *ad libitum*.

Plant materials

A healthy, disease free, plant samples were collected during blooming seasons for each plant, the leaves of *Nerium Oleander* from Apocynaceae family were collected from Delejha region, near Bazyan town/ Sulaimani province in June 2015, and the collection of the bulbs of Narcissus tazetta from Amaryllidaceae family was from Maluma region, near Gapeelon town , Sulaimani province in March 2015 . The leaves and/or the bulbs of the plants were cleaned with water, skinned, chopped and then air-dried indoors at room temperature, the plants authenticated by Prof. Dr. Salim Shahbaz, a taxonomist in Agriculture and Forestry College, Duhok University, Iraq.

Preparation of plant extracts

A modified method of [17] was used for the preparation of the aqueous extracts from *Nerium Oleander* leaves and *Narcissus tazetta* bulbs. In this study we choose the crude extract because traditional medicinal herbs in Iraq are mostly used by human as aqueous extracts. The dried leaves and the dried bulbs were grounded for 15 minutes using electronic grinder and then suspended in distilled water for 24hrs, at the rate of 50gm/200ml. The obtained extracts passed through a sieve (mesh 60), then twice filtered through What man filter paper, ,then the filtrates were concentrated to semi dryness under reduced pressure and controlled temperature (40-47°C) by using rotary evaporator, the residues were put in petri dish and dried in an oven at (37°C) , pooled weighed, kept in dark containers at -4°C. The prepared doses for *Nerium oleander* extract were (92 mg/Kg bw designated as NerD1, 46mg/Kg bw were designated as NerD2 and 23mg/Kg bw designated as NerD3). While For *Narcissus tazetta* the prepared doses were (75 mg/Kg bw designated as NarD1, 37mg/Kg bw designated as NarD2 and 18mg/Kg bw designated as NarD3).

Determination of LD50

The LD50 of the plant extract were determined by the classical method according to Behrens and Karbers [18].The animals were injected intraperitoneally with graded single doses of plant extracts starting with the

highest dose that kill all treated animals reaching the lowest dose at which all treated mice survive. For each dose six mice were used and mortality numbers were recorded after 24 hours.

Experimental design

Three doses of *Nerium oleander*, which were chosen on the basis of the value of LD50, were given intraperitoneally (92 mg/Kg bw designated as NerD1, 46mg/Kg bw were designated as NerD2 and 23mg/Kg bw designated as NerD3) for three groups of laboratory mice each group was consists of ten mice (five mice for chromosomal aberration and mitotic index assay and the other five for micronuclei preparation).

The same doses were given to another three groups of the same number in combination with 15mg/Kg bw of the drug 6-mercaptopurine (The concentration of 6MP was selected according to a previous experimental study in our laboratory that proved its genotoxic ability), Positive control group were given only 6-mercaptopurine intraperitoneally, while untreated mice were considered as negative control, consequently the total number of groups were reached eight groups. The mice were dissected and the data were recorded after 24 hours of treatment. The same design was used for *Narcissus tazetta* bulbs except for extract doses levels, the used doses were (75 mg/Kg bw designated as NarD1, 37mg/Kg bw designated as NarD2 and 18mg/Kg bw designated as NarD3).

Statistical analysis

Statistical analysis of the data were done by using the statistical program SPSS (version 17) to evaluate the effect of different factors on different variables under the study after ANOVA. ($P \leq 0.05$) was considered statistically significant and $P<0.01$ was considered statistically highly significant. Least significant differences (LSD) were used to compare between the means of the treatments that showed significant differences.

Cytogenetic Assays

A- Chromosome Anomalies Assay

The chromosomes were prepared by using direct method [19]. By injecting the treated mice intraperitoneally with 1 ml freshly prepared colchicine solution (4-5) hrs. before dissection to collect the marrow cells in metaphase, then the mice were dissected for bone marrow extraction followed by treating with 5ml of hypotonic KCl solution (0.56%), incubated in73°C for 20minutes, after incubation centrifugation at 1000 rpm for 10minutes was carried out. Supernatant was discarded and fresh Carnoy's fixative was added (3:1 Methanol: Acetic acid) then several successive addition of fixative and centrifugations were followed prior to staining by Gimsa and examination of at least 100 metaphase cells per animal for investigation of chromosomal aberrations.

B-Mitotic index (MI) assay

The slides were examined under light microscope with (40X) power, and 1000 of the divided and non-divided cells were counted and the percentage rate was calculated for only the divided ones according to this equation: M.I. %=No. of dividing cells in metaphase / {Total No.of dividing cells +No. of non-dividing cells (1000) cells} X 100 according to Becker [20].

C-Micronucleus assay

Micronucleus test was performed by the method of Schmid [21] with few modifications. Bone marrow cells from both femur of each animal were flushed with 5 ml of human albumin (inactivated in water bath at 70°C for half an hour)the obtained suspension was centrifuged at 1000 rpm for 5 minutes. The supernatant was discarded and one drop of pullet was smeared on a clean slide. The slides left for complete air drying, fixed in methanol for 5 min then stained with Giemsa stain for 15 minutes. A total of 1000 polychromatic erythrocyte were scored in each treated animal from a single slide to determine the frequency of micronucleated cells.

Results and Discussion

1-Toxicity tests (Calculation of LD50)

a-Toxicity of *Nerium oleander* aqueous extract :

Nerium oleander calculated median lethal dose (LD50) in this study was 922.023 mg/kg bw as evealed in Table (l).

Table (1): Estimation of zero and hundred %mortalities in mice treated with different concentration of *Nerium oleander*

Doses mg/Kg bw	No. of treated mice	No. of dead mice	Percent of mortality
1000	6	6	100
990	6	6	100
975	6	5	83
950	6	3	50
925	6	4	67
900	6	1	16
850	6	0	0
800	6	1	16
750	6	0	0
700	6	0	0

The Intraperitoneal administration of *Nerium oleander* aqueous extract had led to some behavioral changes including, restlessness, irregular movements with decreasing responses to outside stimuli , but the mice that finally were died, the death occur within 10-30 minutes which indicate acute toxicity of *Nerium oleander*.

Rapid death of mice or acute toxicity mostly might be due to Oleanderin and neriine the two potent cardiac glycosides (cardenolides) that are found in all parts of the *N. oleander* especially the leaves [22]. Cardiac glycosides are cardenolides that inhibit the cellular membrane sodium–potassium pump (ATPase) [23,24] with resulting depletion of intracellular potassium and its increase in serum [25]. Toxic doses of the glycosides cause a variety of severe dysrhythmias and conduction disturbances through the myocardium that result in decreased cardiac output [26]. Morphological studies of treated cells with oleandrin indicated that its cytotoxicity effect is via inducing of apoptotic death [27].

b-Toxicity of Narcissus tazetta aqueous extract

The calculated median lethal dose (LD50) in this study was 752.083 mg/kg bw, as illustrated in table (2).

Table (2): Estimation of zero and hundred %mortalities in mice treated with different concentration of *Narcissus tazetta*

Doses mg/Kg b.w	No. of treated mice	No. of dead mice	Percent of mortality
975	6	6	100
950	6	6	100
900	6	4	67
850	6	5	83
800	6	4	67
750	6	3	50
725	6	5	83
700	6	0	0
650	6	3	50
600	6	0	0
550	6	0	0

It was noticed that the injected mice, with aqueous extract of *N. tazetta* bulbs, showed some signs of restlessness, fatigue, loss of appetite , and the animals did not died rapidly, but within 20-48 hrs. of injection. All the treated animals have showed anal blockage of a black-colored intestinal output that may represent unabsorbed part of the injected extract combined with intra-intestinal materials.

The causes of toxicity in Narcissus plants has been poorly characterized .so far, alkaloids, masonin, homolycorin and Narcin was found to exhibit allergenic properties [28,29].

2-Chromosome aberration assay

a- The effect of Nerium oleander, alone and when combined with, 6-Mercaptopurin on chromosome aberrations in mice bone marrow cells.

The effect of *Nerium oleander* aqueous extracts were highly significant on chromatid gap, centromeric break and ring chromosome, see Table (3) and Figure (1). The comparison between mean values which are shown in Table (4) demonstrated that the dose 92mg/kg of N. oleander (NerD1) exerted significant effect on centromeric break and ring chromosome compared with that of control (untreated) mice. When combined with 6-MP the dose 92mg/kg N. oleander aqueous extract (NerD1+6MP) significantly increased the mean values of chromatid gap and ring chromosome if compared to positive control.

These data indicated that *N. oleander* showed genotoxic effect and has the ability to induce chromosomal aberrations like chromatid gap, centromeric break and ring chromosome. No studies had found to agree or disagree with the results of the present study in respect with the effect of *Nerium oleander* aqueous extracts or its combination with 6MP on chromosome aberrations.

Table(3):Analysis the variance for the effect of *Nerium Oleander* and *Narcissus tazetta* extracts and their differences when combined with 6-Mercaptopurine on chromosome aberrations in bone marrow cells of male albino mice

Chromosome aberrations / Factors	Degree of freedom	Mean Squares						
		Chromatid Break with Fragment	Chromatid Break without Fragment	Chromatid Gap	Centromeric Break	Centromeric Gap	Ring Chromosome	Dicentric Chromosome
Nerium oleander (Ner)	7	1.539	1.768	5.300**	15.986**	2.025	26.454**	1.657
Error	32	0.925	0.987	1.188	3.163	2.163	3.100	2.075
Narcissus tazetta (Nar)	7	6.511**	4.111*	10.443**	18.082**	1.586	46.629**	7.139**
Error	32	1.287	1.563	1.438	3.075	2.025	3.238	1.963

The significant levels : $(P < 0.05)^{*}$ $(P < 0.01)^{**}$

b- The effect of Narcissus tazetta, alone and when combined with, 6-Mercaptopurin on chromosome aberrations in mice bone marrow cells.

The values that are shown in Table (3) and Figure (1) demonstrated that *Narcissus tazetta* treated mice, showed significant effect on chromatid break without fragment and highly significant effect on the other chromosomal aberrations like : chromatid break with fragment, chromatid gap, centromeric break, ring chromosome and dicentric chromosome , but the effect of *N. tazetta* treatments on centromeric gap was not significant. The mean values were shown in Table (4) it has been noticed that the dose 75mg/kg of N. tazetta (NarD1) has significant greater mean values comparing with the negative control for most of the chromosomal aberrations: chromatid break with fragment, chromatid break without fragment , centromeric break , ring chromosome .The three experimental doses 75,37,18 g/kg of *N.tazetta* when combined with 6MP lead to significant increase on most chromosomal aberrations like: chromatid break with fragment, chromatid break without fragment, chromatid gap, centromeric break, ring chromosome and dicentric chromosome, mean values were shown in Table (4) .It has been shown that 6-Mercaptopurine itself induces DNA damage, such as single-strand breaks, DNA protein cross-links, interstrand crosslinks, sister chromatid exchanges and chromosome breakage [30], so it's possible that the crude extract of the experimental plants in this study may show additional genotoxicity that enhance the activity of 6MP. Also it appears that *Narcissus tazetta* have the ability to enhance the effect of 6MP more than *Nerium oleander* extracts for most of the chromosomal aberration types.

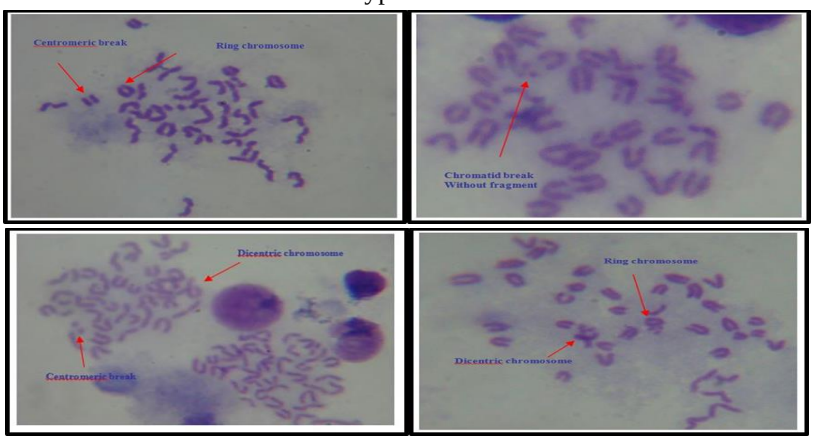

Fig. (1): Types of structural chromosomal aberrations induced by plant extractions and their interaction with 6MP(1000X)

Table (4): Mean ± S.E. for the effect of *Nerium oleander* and *Narcissus tazetta* and their differences when combined with 6-Mercaptopurin on chromosome aberrations in bone marrow cells of laboratory albino mice.

Factors	Chromosome aberrations	Chromatid Break with Fragment	Chromatid Break without Fragment	Chromatid Gap	Centromeric Break	Centromeric Gap	Ring Chromosome	Dicentric Chromosome
Control		0.4 ± 0.219	0.600 ± 0.357	1.2 ± 0.178	1.2 ± 0.521	1.8 ± 0.334	0.6 ± 0.219	0.4 ± 0.219
6-Mercaptopurine(6MP)		0.8 ± 0.521	1.600 ± 0.219	1.4 ± 0.536	3.6 ± 0.536	2.6 ± 0.606	4.8 ± 0.521	1.8 ± 0.769
Nerium oleander (Ner)	NerD1 92mg/kg	1.4 ± 0.219	1.2 ± 0.334	1.2 ± 0.334	4.0 ± 0.400	2.4 ± 0.357	4.2 ± 0.593	1.4 ± 0.456
	NerD2 46mg/kg	0.8 ± 0.334	1.2 ± 0.251	1.2 ± 0.334	1.4 ± 0.456	2.0 ± 0.489	2.4 ± 0.456	1.2 ± 0.521
	NerD3 23mg/kg	0.6 ± 0.219	1.4 ± 0.456	1.2 ± 0.334	1.8 ± 0.438	2.2 ± 0.769	2.0 ± 0.282	1.2 ± 0.657
	NerD1+6MP	1.6 ± 0.456	2.6 ± 0.606	4.0 ± 0.632	5.8 ± 0.954	3.2 ± 0.489	7.4 ± 0.876	2.2 ± 0.657
	NerD2+6MP	1.4 ± 0.219	2.0 ± 0.282	1.8 ± 0.178	3.4 ± 0.829	3.4 ± 0.829	6.2 ± 1.397	2.0 ± 0.565
	NerD3+6MP	2.0 ± 0.632	1.6 ± 0.219	2.8 ± 0.657	5.6 ± 1.523	3.4 ± 0.669	5.4 ± 0.536	1.8 ± 0.593
	LSD	1.242	1.283	1.407	2.296	1.899	2.273	1.860
Narcissus tazetta (Nar)	NarD1 75mg/kg	2.0 ± 0.400	2.0 ± 0.282	0.8 ± 0.334	4.4 ± 0.400	2.6 ± 0.219	5.2 ± 0.912	1.4 ± 0.456
	NarD2 37 mg/kg	2.0 ± 0.565	2.0 ± 0.565	1.4 ± 0.357	5.2 ± 0.593	2.8 ± 0.334	3.2 ± 0.334	0.8 ± 0.334
	NarD3 18 mg/kg	1.6 ± 0.357	1.4 ± 0.606	1.0 ± 0.282	4.0 ± 0.400	2.0 ± 0.400	4.0 ± 0.979	1.6 ± 0.606
	NarD1+6MP	3.0 ± 0.400	3.4 ± 0.536	4.6 ± 0.456	6.8 ± 0.521	3.6 ± 1.080	9.8 ± 0.912	4.0 ± 0.489
	NarD21+6MP	3.6 ± 0.669	3.0 ± 0.489	3.0 ± 0.632	6.8 ± 1.109	2.2 ± 0.178	8.0 ± 0.938	3.2 ± 0.912
	NarD3+6MP	3.2 ± 0.334	2.6 ± 0.726	3.8 ± 0.769	6.2 ± 1.073	2.8 ± 0.769	8.4 ± 0.456	2.2 ± 0.334
	LSD	1.465	1.614	1.548	2.264	1.837	2.323	1.809

3- Mitotic index and Micronuclei Assay

a- The effect of *Nerium oleander* alone and when combined with 6-Mercaptopurin on mitotic index and total micronucleated cells in mice bone marrow cells.

Nerium oleander extract in Table (5) showed a highly significant effect (P≤0.01) on mitotic index. The results in Table (6) showed that the doses 92g/kg and 46g/kg of *N. oleander* (NerD1 & NerD2) aqueous extracts significantly decreased the mitotic index comparing with control ,while the reduction in mitotic index that caused by the lowest dose 23mg/kg (NerD3) was not significant. Similar results were observed by Wong [31] and Raghavendra [32] when the leaves extracts of *N. oleander* displayed antiproliferative activity against some cancer cell lines. This result is also agreed with the findings of Peiying [23] that determine the clastogenic and cytotoxic activity of oleandrin (an active component of *Nerium oleander* extract which inhibited the proliferation of rodent and human pancreatic cancer cell lines.Table (6) also showed that all the three doses of *Nerium oleander* aqueous extracts in combination with 6MP caused a significant decrease in mitotic index if compared with control (untreated mice) and non-significant decrease if compared with positive control (6MP).

The results of this study showed that *N. oleander* itself is a potent anti-mitotic agent which is agree with some previous studies [31,32,33] and when combined with 6MP it can enhance its antimitotic activity but not significantly. *Nerium oleander* aqueous extracts alone or when combined with 6MP showed non-significant effect on total micronucleated cells, as shown in Table (5) and figure (2). The Table of means (6) showed this result except for the dose 92 mg/kg of *Nerium oleander* (NerD1) which show slightly significant increase in total micronucleated cells, the other doses and even the combination treatments with 6MP did not significantly changed the total number of bone marrow micronucleated cells. No reports have been recorded indicating the effect of N. oleander on micronucleus in bone marrow cells of mice.

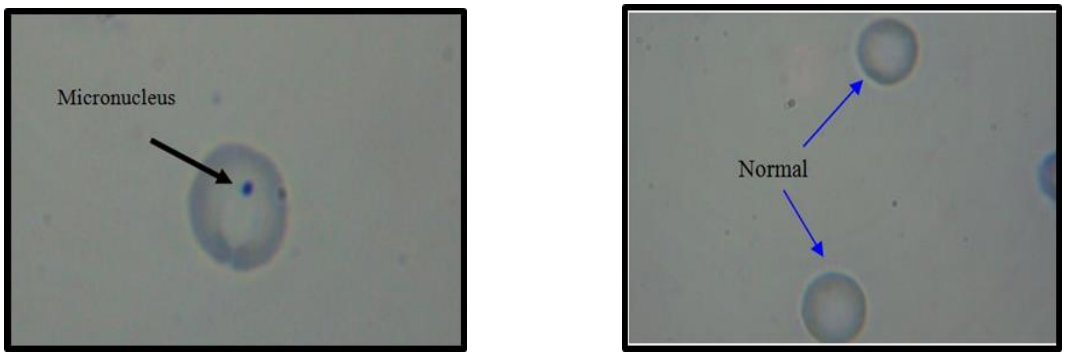

Fig. (2): Micronucleated bone marrow cells induced by plant extractions and their interactionwith 6-Mercaptopurine

Table (5): Analysis of variance for the effect of *Nerium oleander* and *Narcissus tazetta* each alone and their differences when combined with 6-Mercaptopurin on mitotic index and total micronucleated cells in bone marrow of male albino mice.

Cytogenetic Assays / Factors	Degree of freedom	Mean Squares	
		Mitotic index %	Micronuclei/ Cell %
Error	32	2.721	1.463
Nerium oleander (Ner)	7	26.335**	1.257
Error	32	1.911	1.238
Narcissus tazetta (Nar)	7	23.477**	3.071*
Error	32	0.965	1.175

b- The effect of *Narcissus tazetta* alone and when combined with 6-Mercaptopurin on mitotic index and total micronucleated cells in mice bone marrow cells.

The ANOVA Table (5) cleared that of *Narcissus tazetta* extract had highly significant effect ($P \leq 0.01$) on mitotic index and significant effect ($P \leq 0.05$) on marrow total micronucleated cells. The Table of mean values (6) demonstrated that all the three used doses of *N. tazetta* had led to reduction in mitotic index compared with untreated control. The combination between the three doses of *N. tazetta* aqueous extract with 6-mercaptopurine significantly decreased the percentage of mitotic index compared with that of positive control (6MP). The present study result of antimitotic property of *N. tazetta* extract, this is agree with James[34], Christophe [35] and Kornienko & Evidente [36] they reported that *N. tazetta* possesses potent antimitotic properties and anticancer effects. Additionally *N. tazetta* has been shown to contain pancratistatin with angiogenesis/ antivascular properties due to protein biosynthesis inhibition in the vascular endothelial cells or is due to an entirely different mode of action [36].

Table (6): Mean ± S.E. for the effect of *Nerium oleander* and Narcissus *tazetta* each alone and their differences when combined with 6-Mercaptopurin on mitotic index and total micronucleated cells in bone marrow of male albino mice

Cytogenetic Assays / Factors		Mitotic index%	Micronuclei/Cell%
Control		16.54± 0.894	0.4 ± 0.219
6-Mercaptopurine (6MP)	15mg/kg	11.50± 0.209	1.4 ± 0.456
Nerium oleander (Ner)	NerD1 92mg/kg	13.90 ± 0.425	2.0 ± 0.282
	NerD2 46mg/kg	14.10 ± 0.714	1.0 ± 0.282
	NerD3 23mg/kg	15.68 ± 0.311	1.0 ± 0.489
	NerD1+6MP	10.52 ± 0.326	1.8 ± 0.567
	NerD2+6MP	11.12 ± 0.823	1.4 ± 0.357
	NerD3+6MP	11.20 ± 0.172	1.4 ± 0.606
	LSD	1.785	1.436
Narcissus tazetta (Nar)	NarD1 75mg/kg	10.76 ± 0.156	2.2 ± 0.178
	NarD2 37mg/kg	11.48 ± 0.217	1.8 ± 0.521
	NarD3 18mg/kg	11.04 ± 0.402	0.8 ± 0.334
	NarD1+6MP	09.68 ± 0.124	2.8 ± 0.593
	NarD21+6MP	10.26 ±0. 293	2.2 ± 0.521
	NarD3+6MP	10.12 ± 0.237	1.6 ± 0.456
	LSD	1.258	1.399

Table (6) also elucidated that both 75mg/kg and 37 mg/kg doses of of *N. tazetta* extract caused significant increase of the total micronucleated bone marrow cells compared with untreated control group. The lowest dose 18 mg/kg also increased the total micronucleated cells, but the change in the frequency is not significant compared with untreated control. No reports have been recorded indicating the effect of *N. tazetta* on micronucleus in bone marrow cells of mice, so this study might be the first report to declare that. When the aqueous extract of *Narcissus tazetta* was combined with 6MP, only the dose 75mg/kg (NarD1+6MP) led to significant increase in the frequency of the total micronucleated cells compared with that of positive control (6MP). A significant increase (compared with control) in the frequency of total micronucleated cells also have been recorded by the other two doses combined with 6MP, but there were no significant differences with the positive control.

By comparing the biological effects of the two plant extracts on mice bone marrow cells, The results showed that Narcissus bulbs extract were more efficient in enhancing the genetic toxicity of 6MP than *Nerium oleander* extract as it increased different types of structural chromosome disorders and increased the total number of micronuclei in addition to its ability to reduce the number of dividing bone marrow cells when given to mice alone or with combination with the drug 6MP.

On the basis of the results obtained in the present study, causing cytotoxicity and genotoxicity by the experimental plant extracts ,mostly *N. tazetta* , alone and together with 6-Mercaptopurine in bone marrow cells of albino mice, indicated that the extracts may have potentially significant implications in the discovery of new plant sources for their anticancer properties (Cytotoxic and genotoxic effects) but we suggest more additional studies to find the exact mixed combination of bioactive component in the crude extract and their exact doses to be combined with the antileukemic drug 6MP in order to enhance its anticancer effect, as well as decrease the resistance to this conventional drug by tumor cells.

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