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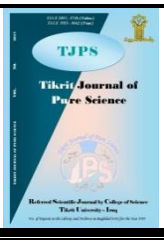

Genotoxic and Cytotoxic Effects of Belomycin and Crud Extracts of Mirabilis jalapa Linn in Male White Mice

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ABSTRACT

Genotoxicity and cytotoxicity of Belomycin (BLM) have been evaluated in bone-marroww cells by micronucleus test, as well as the analysis of sperm shape abnormalities in male white mice, considering that BLM is the most wide anticancer drug used with patients. Also, the study includes assessment the effect of crude water and alcoholic extracts of the four o'clock flowers (Mirabilis jalapa Linn) in reducing BLM toxicity and the study was carried out in the Genetics Laboratory of the Department of biology for the period from 1-10-2017 to 1-5-2019.So the genotoxicity and cytotoxicity were evaluated independently and in conjunction between two different dosages of BLM 0.8 and 1.6 $mg.kg^{-1}$.bwt. and three orally dosage of different concentration of crud extracts, which is 39.8, 26.52, 13.26 $mg.kg^{-1}$ and 7.02, 4.68, 2.34 $mg.kg^{-1}$ o water and alcohol extract respectively.

The results of assessment of BLM genotoxic effects showed that the drug caused induction of micronuclei, here were significant increase in micronucleated polychromatic erythrocytes (MNIPCEs) and significant increase in micronuclei(MNI) in the groups treated with 0.8 and 1.6 $mg.kg^{-1}$ of BLM, compare to negative control at the level of significance $P < 0.05$

On the other hand, the results showed that BLM has potential to induce sperm shape abnormalities, which include head and tail abnormalities, It included an increase in the proportion of morphological abnormalities in the head and tail of the sperm when compared to negative control at the significant level of $P < 0.05$.

The results also showed, that treatment with low dosages of four o'clock flower crud extracts didn't induce neither micronuclei or any increase in PCEs numbers nor sperm shape abnormalities, although some toxic effects do exist with the higher dosages.

Evaluation of results from dependent treatments of BLM and different concentrations of water and alcoholic crud extracts, we observed significant role of these extracts in reducing toxic effects of the drug BLM in bone marrow cells, which caused significant decrease in mean differences of MNIPCEs and MNI. More over the results showed significant decrease in mean differences of sperm shape and tail abnormalities compared to negative control.

Results of the current study suggest that water and alcoholic four o'clock flower crud extracts have a role in reducing genotoxic and cytotoxic effects of BLM in bone-marrow cells and sperms of white mice.

Introduction

Bleomycin is considered a glycopeptide antibiotic derived from the bacterium *Streptomycin verticillius* which has an anti-tumor and anticancer effect and is widely used in chemotherapy systems for various types of cancers such as lymphoma and squamous cells in the head and neck, and is an adjunct to radiation therapy by inducing apoptosis in tumor cells and sniping transformed and cancer cells [1]. It also has the ability of solubility in water and used for intramuscular injection [2]. Despite the positive role of Bleomycin in stimulating tumor cell death, but it directly causes cell toxicity in body cells and organs.

It mediates the therapeutic effects of the level of DNA alteration and stimulates programmed cell death by inhibiting the cell cycle in phase G2 and phase M and works to inhibit protein synthesis and thereby inhibiting the building of amino acids. It also works on the formation of free radicals that cause direct oxidation of cells and cause fractures in single, double and mono-DNA strands, which are manifestations of genetic and cellular toxicity of the drug [3,4].

Medical plants have been used in many studies and research on the role of raw plant extracts in the elimination of toxicity of chemical drugs in living cells. Studies have shown the results of the preventive role of extracts in reducing the effects of toxicity because they possess biologically active substances that perform the physiological work in the body [5,6]. The Mirabilis Jalapa plant is regarded a plant of effective medicinal value in the fields of research and treatment of many diseases [7]. Due to the lack of studies in this field, the present study was designed to determine the effectiveness of the raw extracts of the Mirabilis Jalapa plant flowers in removing the cytotoxic and genotoxic effects of Bleomycin.

Materials and Methods

Method of preparation and extraction of plant material

The Mirabilis Jalapa plant flowers were obtained from the home gardens of Al-Alam district and diagnosed in the College of Agriculture / University of Tikrit, where the genus and species were determined based on the diagnostic characteristics of M. Jalapa plant. Linn. Its flowers were taken and left to dry at room temperature 30c away from direct sunlight for a week and then grinded by an electric mill and converted to a soft powder to carry out the extraction [8].

Method of Extraction

The raw water and alcohol extract was prepared using the Soxhlet device, in which 100 gm of floral powder was taken and placed in the bag of extraction inside the device. Then, 500 ml of water was added regarding the water extract and 500 ml of methanol was added in the case of the alcohol extract. After 72 hours of extraction, the solvent was removed using a rotary evaporator and obtaining the raw extract in a form of dark brown material that can be kept in dark and sterile glass bottles in a cool place until use [9, 10].

Experimental Groups

In this experiment, treatments were divided into 22 for the two tests of micronuclei and sperm abnormalities with 5 animals for each group. And the study was carried out for the period from 1-10-2017 to 1-5-2019, Treatments were conducted via intravenous injection using veterinary syringes or oral infusion and performed using a syringe that was modified for this purpose as shown below:

1- The negative control group was treated with normal saline solution.

2- The positive control group was given 20 $mg.kg^{-1}$. body weight of cyclophosphamide drug.

3- Three groups were given the first Bleomycin dose 0.8 $mg.kg^{-1}$and the second dose 1.6 $mg.kg^{-1}$.

4- Three groups of mice were given each 39.78 ,26.52 and 13.26 $mg.kg^{-1}$ of the water extract of Mirabilis Jalapa plant flowers by oral administration.

5- Three groups of mice were given each 7.02 , 4.68 and 2.34 $mg.kg^{-1}$ of the alcohol extract of Mirabilis Jalapa plant flowers by oral administration.

6- Three groups of mice were each given 0.8 $mg.kg^{-1}$ of Bleomycin drug in the membrane with 39.78,26.52 and 13.26 $mg.kg^{-1}$ of the water extract of Mirabilis Jalapa plant flowers by oral administration.

7- Three groups of mice were each given 0.8 $mg.kg^{-1}$ of Bleomycin drug in the membrane with 7.02 , 4.68 and 2.34 $mg.kg^{-1}$ of the alcoholic extract of Mirabilis Jalapa plant flowers by oral administration.

8- Three groups of mice were each given 1.6 $mg.kg^{-1}$ of Bleomycin drug in the membrane with 39.78,26.52 and 13.26 $mg.kg^{-1}$ of the water extract of Mirabilis Jalapa plant flowers by oral administration.

9- Three groups of mice were each given 1.6 $mg.kg^{-1}$ of Bleomycin in the membrane with 7.02 , 4.68 and 2.34 $mg.kg^{-1}$ of alcohol extract of Mirabilis Jalapa plant flowers by oral administration.

1 - Determination of micronuclei in immature red blood cells(PCEs) in bone marrow of white male mice:

The animals were subjected to separation of cervical vertebrae after 18 hours from the time of oral gavage of the negative and positive control groups treated with Bleomycin drug and treated with water and alcohol extracts individually, then by joint treatment and according to the design of each experiment. The test was performed according to the method described [11]. The prepared slides were examined using a standard optical microscope and under 40X magnification. One thousand blood cells per animal were calculated, investigation of presence of PCEs was conducted and MNIPCEs and MNI were calculated.

2 - Determination of morphological abnormalities in the white male mice sperms

The animals were subjected to separation of cervical vertebrae after 35 days of treatment for the negative and positive control groups treated with Belomycin and treated with water and alcohol extracts independently and then jointly according to the design of each experiment. The test was then conducted as described in [12].

Statistical Analysis

The statistical package for Social Sciences (SPSS) was used for statistical analysis at a probability level ($P \leq 0.05$), using ANOVA variance analysis. The highest differences were estimated by the Tukey test.

Results and Discussion

Micronuclei Test Estimation

Figure (1) shows immature red blood cells (polychromatic erythrocytes), and micronucleated polychromatic erythrocytes in the bone marrow of the mice in the negative control group, positive control group and various treatments.

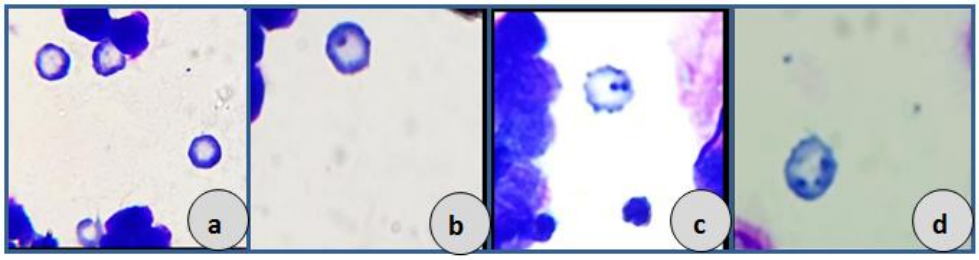

Fig. 1: micronuclei in immature red blood cells in the bone marrow of the white mice after treatment with Bleomycin drug.It shows a- polychromatic erythrocytes ,b- polychromatic erythrocytes containing one micronuclei, c- polychromatic erythrocytes containing two micronuclei d- polychromatic erythrocytes containing three micronuclei *n=40. may-grunwald +Gemsa.(100X*).

Table (1) shows mean differences values and standard mean error for the number of micronucleated PCEs and the number of micronuclei for different treatments of BLM, cyclophosphamide, water extract, and an alcoholic extract compared to the values of the negative control group .at a Significant level (P≤ 0.05).

Table 1: Mean differences values for number of MNIPCEs and MNI for different treatment groups

N	Treat. Mg.kg^{-1}.b.wt	Negative control D.W Group number	MNIPCEs MD ± S.E	MNI MD ± S.E
2	CP 20	1	1.25 ± 9.00 *	1.85 ± 14.20 *
3	0.8 BLM	1	1.25 ± 26.00 *	1.85 ± 42.80 *
4	BLM 1.6	1	1.25 ± 26.00 *	1.85 ± 48.60 *
5	E .w 39.78	1	1.25 ± 5.80	1.85 ± 11.20 *
6	E .w 26.52	1	1.25 ± 4.20	1.85 ± 9.00
7	E .w 13.26	1	1.25 ± 0.80	1.85 ± 2.60
8	E.ch 7.02	1	1.25 ± 4.40	1.85 ± 8.80
9	E.ch 4.68	1	1.25 ± 2.60	1.85 ± 6.80
10	E.ch 2.34	1	1.25 ± 2.20	1.85 ± 5.00
11	BLM 0.8 + E .w 39.78	1	1.25 ± 4.00	1.85 ± 5.80
12	BLM 0.8 + E .w 26.52	1	1.25 ± 1.60	1.85 ± 2.80
13	BLM 0.8 + E .w 13.26	1	1.25 ± 0.40	1.85 ± 0.00
14	BLM 1.6 + E .w 39.78	1	1.25 ± 3.00	1.85 ± 4.20
15	BLM 1.6 + E .w 26.52	1	1.25 ± 1.80	1.85 ± 2.26
16	BLM 1.6 + E .w 13.26	1	1.25 ± 2.00	1.85±2.00
17	BLM 0.8 + E.ch 7.02	1	1.25 ± 3.60	1.85 ± 4.80
18	BLM 0.8 + E.ch 4.68	1	1.25 ± 3.20	1.85 ± 5.40
19	BLM 0.8 + E.ch 2.34	1	1.25 ± 2.00	1.85 ± 3.60
20	BLM 1.6 + E.ch 7.02	1	1.25 ± 3.60	1.85±4.60
21	BLM 1.6 + E.ch 4.68	1	1.25 ± 3.00	1.85 ± 4.00
22	BLM 1.6 + E.ch 2.34	1	1.25 ± 3.20	1.85 ± 3.80

*Significant on level (p≤ 0.05), schefee test, cp :Cyclophospamid, BLM : Bleomycin, E :extract W :Water, Ch : chohol**.**

The results in Fig. (1) and Table (1) show a significant increase in mean differences of the numbers of micronucleated polychromatic erythrocyte (MNIPCEs) and MNI when treated with Bolemycin drug with doses 0.8 mg.kg^{-1} and 1.6 mg.kg^{-1} . This can be attributed to the high efficacy of the drug for cell binding, direct oxidation and the formation of free radicals inside and outside the cells

leading to chromosomal fractures in a path not supported by creation of DNA S-independent clastogen or by inhibiting the creation of DNA and RNA and formation of protein, and forming complexes with molecular oxygen linked to DNA and separation of DNA stripes. The increase in micronuclei repetition refers to the high levels of genetic damage that can lead to mutations [15,14,13] . Such cytotoxic effects of BLM have been indicated in increasing the number of micronuclei in human lymphocytes as reached by [16].

The results of the groups treated with water and alcohol extracts for Mirabilis Jalapa plant flowers also showed that there were no significant differences in the mean values of the average number of micronucleated polychromatic erythrocyte (MNIPCE) or the average micronuclei (MNI). This indicates that the doses used in this study had no toxicity effects on the induction of forming micronuclei. The results of the joint treatments between Bolemycin drug and water and alcoholic extracts were accompanied by a significant decrease in the mean difference values for the number of micronucleated polychromatic erythrocyte , and the number of micronuclei in bone marrow cells when compared to the drug-treated groups alone. It is concluded that water and alcohol extracts for Mirabilis Jalapa plant flowers have a role in reducing the toxic effect of Bolemycin drug in bone marrow cells. This can be attributed to the fact that extracts contain biochemicals such as flavonoids, tannins and phenols, which are able to remove the toxicity of the drug by preventing their ability to snipe free radicals, prevent oxidation within cells, terminate free chain reaction, protect cells from oxidation, and thus protect the structure of DNA [17,6]. This is confirmed by research studies on the role of water and alcohol extracts of plants in inhibiting the genotoxicity and cytotoxicity of Bolemycin drug in human lymphocytes and pulmonary endothelial cells [18, 19].

Testing the estimation of morphological abnormalities in mature sperms

Figure (2) shows the shape of the sperm in its natural state, the types of morphological abnormalities caused by the different treatments in the negative control group and the positive control group (cyclophosphamide), the doses 0.8 and 1.6 mg.kg-1 of BLM drug, different concentrations of water and alcohol extracts of *M. jalapa linn* plant flowers, and joint treatment with BLM drug and various concentrations of water extract and alcohol extract.

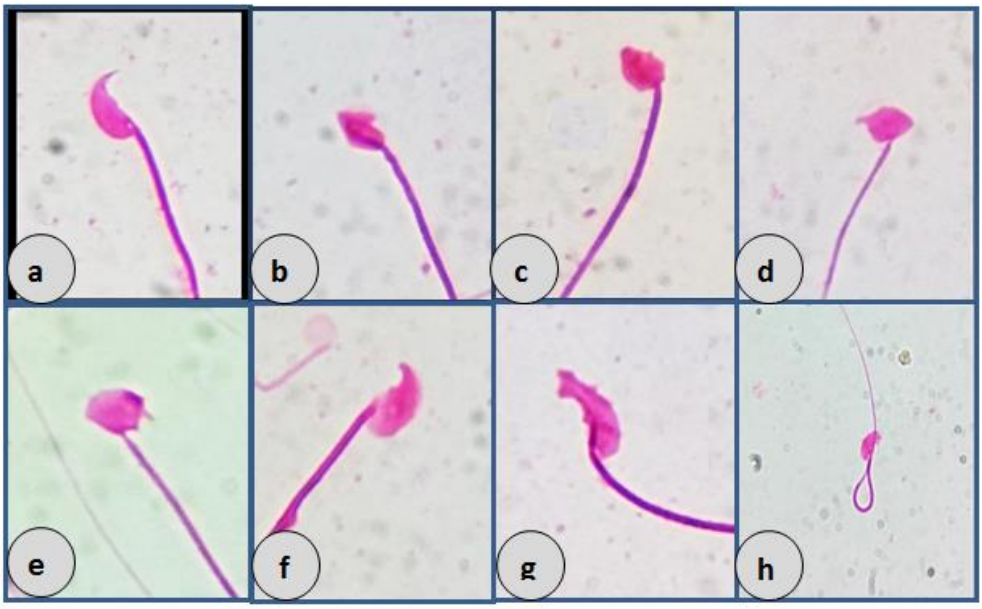

Fig. 2: Sperms abnormalities induced by one treatment with Bolemycin a- normal head, b-triangular head, c- without hook, d- irregular head, e- spherical head, f- large head, g- banana head, h- coiled tail of Eiosin,(X100).

Table (2) shows the mean difference values for the total abnormalities in the head area, the total tail abnormalities of the mature sperm and the rates of types of abnormalities in sperm head of mice in the group treated with cyclophosphamide drug (positive control), BLM drug, different concentrations of the water extract and the alcohol extract of *M, jalapa. Linn* plant flowers, and different concentrations of the two extracts.

Table 2: Mean difference values of total head and tail abnormalities of mature sperm and rates of types of abnormalities in adult sperm head for different treatment groups of white mice

N	Treat. $Mg.kg^{-1}.b.wt$	NC. D.W Group .N	Head without hook %	Irregular head %	Large head %	Small head %	Triangle head %	Spherical head %	Head like banana %	Head sperm MD ± S.E	Tail sperm MD ± S.E
2	CP 20	1	19.0	32.0	2.3	27.8	0	0	17.8	7.44 ± 29.8 *	44.80 ± 44.8
3	0.8 BLM	1	3.6	52.8	18.9	2.1	4.3	4.9	13.1	7.44 ± 279.6 *	15.66 ± 67.8 *
4	BLM 1.6	1	2.8	52.4	13.2	17.2	6.2	0.8	7.0	7.44 ± 254.6 *	15.66 ± 98.6 *
5	E .w 39.78	1	10.5	60.4	1.6	10.4	9.1	0.2	7.5	7.44 ± 164.6 *	15.66 ± 44.8
6	E .w 26.52	1	13.2	44.6	2.3	16.1	14.4	0.4	8.7	7.44 ± 77.0 *	15.66 ± 63.8 *
7	E .w 13.26	1	10.6	63.6	1.7	12.4	3.2	0	8.1	7.44 ± 52.6 *	15.66 ± 80.8*
8	E.ch 7.02	1	8.6	50.8	6.3	18.1	0	0.4	15.5	7.44 ± 140.6*	15.66 ± 134.6*
9	E.ch 4.68	1	10.8	40.1	10.1	23.1	0	0	15.6	7.44 ± 50.8*	15.66 ± 81.4 *
10	E.ch 2.34	1	4.2	42.3	5.3	24.2	0	0	23.8	7.44 ± 19.0	15.66 ± 22.8
11	BLM 0.8+E .w39.78	1	2.6	39.1	0	36.6	0	0	21.5	7.44 ± 63.0*	15.66 ± 69.6 *
12	BLM 0.8+E .w26.52	1	1.8	35.6	0	38.4	0	0	24.1	7.44 ± 28.6 *	15.66 ± 87.8 *
13	BLM 0.8+E .w13.26	1	0.6	30.2	0	36.2	0	0	32.8	7.44 ± 7.4	15.66 ± 96.4 *
14	BLM 1.6+E .w39.78	1	5.6	60.5	7.9	14.2	1.1	0.4	10.0	7.44 ± 146.2 *	15.66 ± 62.20 *
15	BLM 1.6+E .w26.52	1	6.9	59.2	8.8	13.6	1.1	0	10.3	7.44 ± 85.6 *	15.66 ± 75.2 *
16	BLM 1.6+E .w13.26	1	5.6	64.5	5.8	13.2	0	0	10.7	7.44 ± 56.2 *	15.66 ± 90.6 *
17	BLM 0.8+E.ch 7.02	1	7.1	42.0	0	37.4	0	0	13.3	7.44 ± 60.0 *	15.66 ± 3.0
18	BLM 0.8 +E.ch 4.68	1	4.8	36.2	0	41.6	0	0	17.2	7.44 ± 3.2	15.66 ± 10.2
19	BLM 0.8 +E.ch 2.34	1	3.6	29.3	0	46.0	0	0	20.9	7.44 ± 32.6*	15.66 ± 51.0 *
20	BLM 1.6+E.ch 7.02	1	5.5	40.1	1.2	26.6	0	0	26.4	7.44 ± 55.0 *	15.66 ± 74.8*
21	BLM 1.6+E.ch 4.68	1	4.8	27.5	0	34.4	0	0	25.3	7.44 ± 20.8	15.66 ± 71.8*
22	BLM 1.6+E.ch 2.34	1	2.6	35.7	0	33.6	0	0	27.8	7.44 ± 5.2	15.66 ± 46.6

*Significant on level ($p \leq 0.05$), Tukey test, cp :Cyclophospamid, BLM : Bleomycin, E :extract, W :Water, Ch : chohol

The results of the present study showed that Bolemycin drug has the potential to induce morphological abnormalities in mature sperms in the head and tail areas as shown in Table (2) and Figure (2). There was a significant increase in mean differences for the number of abnormal sperm. This is due to the role of BLM drug in the creation of direct genotoxicity and cytotoxicity through participation in oxidation-reduction reactions and the formation of free radicals such as hydroxyl, which can interact with cells and composition of lipid peroxidation, which in turn has the ability to react with cells and form fat peroxide that destroys cell membranes and damages DNA, and the possibility of mutations in sperm and the subsequent genetic state of posterity [20, 21]. This result is similar to that of [22,23]. The toxicity of Belomycin was studied extensively and was found to cause oxidative stress, induced apoptosis, inhibition of ATP In the mitochondria, low energy output of the sperm , as well as decrease in mitochondria membrane potential that leads to the launch of Cytochrome C and the death of sperm cells [24].

The study of the results of treatment groups with water and alcohol extracts of Mirabilis Jalapa plant flowers found some differences in the level of morphological abnormalities of the sperms at high doses, and this may be due to the role of some compounds found in extracts such as alkaloids, which can cause cellular toxicity in sperms. This is what was referred to by the study of [25] on the toxic effect of alkaloids in sperm cells by binding sections of the DNA bases with each other or those adjacent to them and the creation of joints in them. From the observation of the results, we find that the lower the dose used, the less the morphological abnormalities in the sperm. This allows for the study of lower concentrations that give a protective role from the toxicity of BLM without side effects.

When evaluating the results of the joint treatment with BLM drug and raw water and alcohol extracts, there was a significant decrease in the mean differences of the total morphological abnormalities of the sperms when compared to the drug only treated groups. This is due to the protective role of the extracts in reducing the toxicity of belomycin. This may be attributed to the fact that these extracts contain antioxidants (flavonoids, phenol acids, and xanthan pigments), their role in free radical snipping and preventing oxidative stress and composition of lipid peroxidation and reduction of DNA damage and fragmentation of sperm cells [26]. The positive role of water and alcohol extracts for Mirabilis Jalapa plant flowers can be explained by the reduction of toxic effect and morphological abnormalities. These extracts can stimulate the formation of the enzyme *Bleomycin hydrolase* to provide a protective role for sperms as it metabolizes BLM molecules into non-toxic molecules and disrupt its role before entering the nucleus and increase the resistance of cells towards it [27, 28, 29].

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تأثير المستخلصات الخام للنبات Mirabilis jalapa في السمية الوراثية والسمية الخلوية للعقار Belomycin في الفئران البيض

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الملخص

تم تقييم السمية الخلوية والوراثية لجرعات مختلفة من عقار البليومايسين BLM في خلايا نقي العظم باستخدام اختبار النوى الدقيقة, فضلا عن تحليل تشوهات الشكل المظهري النطف الناضجة باعتباره من أكثر المضادات الحيوية المستخدمة على نطاق واسع لمعالجة مرضى السرطان, كما تضمنت الدراسة تقدير اثر المستخلصات المائية والكحولية لأزهار نبات الساعة الرابعة ***Mirabilis Jalapa*** في خفض التأثيرات السمية التي يحدثها عقار البليومايسين, لذاك تم تقييم السمية الوراثية والخلوية للعقار والمستخلصات بشكل مستقل ثم تقييم التأثير المشترك للعقار مع تراكيز مختلفة من المستخلصين المائي والكحولي ولقد أنجزت الدراسة في مختبر الوراثة قسم علوم الحياة للفترة من 1-10-2017 إلى 1-5-2019.

تم حقن الحيوانات بالجرعات 0.8 و1.6 ملغم.كغم$^{-1}$ من عقار البليومايسين لمرة واحدة ولمدة 24 ساعة, وتمت التضحية والتشريح في اليوم الثاني لاختبار النوى الدقيقة وبعد 35 يوماً من المعاملة لاختبار التشوهات المظهرية في النطف الناضجة وبالجرعات 39.78 و 26.52 و 13.26 ملغم.كغم$^{-1}$ للمستخلص المائي وبالجرعات 7.02 و68 .4 و2.34 ملغم.كغم$^{-1}$للمستخلص الكحولي عن طريق التجريع الفموي.

أظهرت نتائج تقدير التأثيرات السمية الوراثية لعقار البليومايسين انه تسبب في حث تكوين النوى الدقيقة في خلايا نقي العظم, إذ تبين حصول زيادة معنوية في كريات الدم الحمر غير الناضجة الحاوية على النوى الدقيقة MNIPCEs وزيادة معنوية في النوى الدقيقة MNI للمجاميع المعاملة بالجرعات 0.8 و1.6 ملغم.كغم$^{-1}$ من عقار البليوميسين مقارنة بالسيطرة السالبة عند مستوى معنوية $P<0.05$. كما وأظهرت نتائج الدراسة امتلاك عقار BLM قدرة على حث التشوهات المظهرية في النطف الناضجة, تضمنت زيادة في نسبة التشوهات المظهرية في رأس وذيل النطفة عند مقارنتها بالسيطرة السالبة عند مستوى معنوية $P<0.05$.

كما وبينت الدراسة أن المعاملة بالجرعات المنخفضة من المستخلصات المائية والكحولية لأزهار نبات الساعة الرابعة لم تترافق مع تأثيرات في حث تكون النوى الدقيقة وزيادة أعداد كريات الدم الحمر غير الناضجة, أو التشوهات المظهرية للنطف الناضجة مع وجود بعض التأثيرات السمية مع الجرعات العالية.

وعند تقييم نتائج المعاملة المشتركة بالعقار BLM والمستخلصات المائية والكحولية, تبين أن المستخلصات أظهرت دوراً كبيراً في الحد من الآثار السمية للعقار في خلايا نقي العظم, إذ تسببت في حصول انخفاض معنوي في متوسطات الفروق لأعداد كريات الدم الحمر غير الناضجة والنوى الدقيقة, كذلك أظهرت النتائج حصول انخفاض معنوي في قيم متوسطات الفروق لتشوهات الرأس والذيل للنطف في المجموعات المعاملة مقارنة بالسيطرة السالبة.

تفترض نتائج الدراسة الحالية أن للمستخلصات المائية والكحولية الخام لأزهار نبات الساعة الرابعة دورا في الحد من التأثيرات السمية الوراثية والخلوية لعقار BLM في خلايا نقي العظم عند الجرعات المستخدمة والنطف الناضجة للفئران البيض.