RESEARCH ARTICLE

The potential antimutagenic effect of Saudi moringa oleifera (*Moringa peregrina*) leaves extract on the bone marrow of male albino mice

Najiah M. Alyamani*

Department of Biology, College of Science, University of Jeddah, Jeddah 21493, Saudi Arabia

Received: March 1, 2023; **accepted:** March 20, 2023.

Researchers have confirmed that the administration of chemotherapeutic drugs can induce genotoxicity which may cause the inhibition of hematopoiesis or damage of the somatic cells due to the genotoxicity and cytotoxicity. Endoxan, a cyclophosphamide, has been used to treat different types of cancer. Moringa Oleifera is a tree native to South Asia but is now grown in many areas of the world due to its numerous health benefits. This study was to investigate the anti-genotoxicity of Moringa Oleifera (*Moringa peregrina*) leaves extract (MLE) on reducing the toxicity of Endoxan by using genotoxic assays that were simple, accurate, and economical for the assessment of the damage of genetic material. 30 Swiss albino male mice (*Mus musculus*) MFI strain were involved in the study to evaluate the genotoxic responses and the effects of MLE against Endoxan through chromosomal aberrations (CA) frequency in bone marrow cells and micronucleated polychromatic erythrocytes (MNPCEs). The results showed that the CA frequency of bone marrow cells and MNPCEs on micronucleus assay increased significantly during the administration of therapeutic dose of Endoxan. On the other hand, all types of dual treatment with MLE and Endoxan demonstrated alleviated genotoxicity by reducing CA and MNPCEs. The results confirmed MLE's capability to reduce Endoxan's cytotoxic and genotoxic effects, which might be explained by the MLE's strong antioxidant properties. The present finding indicated that MLE demonstrated anti-genotoxic potential on the toxicity of chemotherapeutic agents. Further toxicity studies are required for confirmations.

Keywords: chromosomal aberrations; Endoxan; *Moringa peregrina*; molecular genetics; bone marrow.

***Corresponding author:** Najiah M. Alyamani, Department of Biology, College of Science, University of Jeddah, Jeddah 21493, Saudi Arabia. Email: nmalyamani@uj.edu.sa.

Introduction

The DNA molecule, which is responsible for genetic makeup, is constantly exposed to various mutagenic and carcinogenic substances that can cause harmful reactions within the body. These substances, such as free radicals, can react with the DNA components, ultimately destroying purine and pyrimidine bases, leading to numerous health complications [1, 2]. Chemotherapy medications are often used to treat cancer, including the chemotherapy agent cyclophosphamide. However, these medications can have unpleasant side effects, and malignant cells can become resistant to them over the time, making them less effective [3]. Cyclophosphamide, in particular, has been found having side effects on the reproductive system including damages to the ovaries and testicles, which can lead to infertility [4]. Despite the potential side effects, chemotherapy remains an important tool in the fight against cancer and is often used in combination with other treatments to achieve the best possible outcomes for patients.

Endoxan (cyclophosphamide) is a chemotherapy drug used to treat a variety of cancers including leukemia, lymphoma, breast cancer, and ovarian cancer. It works by slowing or stopping the growth of cancer cells. Like all chemotherapy drugs, Endoxan can cause side effects, some of which can be severe. The most common side effects of Endoxan include nausea and vomiting, hair loss, loss of appetite, diarrhea, mouth sores, and fatigue. Endoxan can also cause more serious side effects including suppression of the immune system. Endoxan can decrease the number of white blood cells in the body, which can increase the risk of infections and damage to the bladder. Endoxan is broken down in the liver and excreted through the urine, which can cause damage to the bladder lining, leading to inflammation and bleeding. Endoxan can damage the ovaries and testicles, leading to infertility. There is a small risk that Endoxan may increase the risk of developing secondary cancers such as leukemia.

Using biological agents to treat cancer instead of chemotherapy, which has fewer or no negative side effects, has grown significantly in recent years. It is estimated that 80% of patients worldwide rely on them for some aspect of their primary healthcare. Alternative natural products include the 13 species of *Moringa peregrina* (*Moringaceae*) from tropical and subtropical climes, which range in size from tiny plants to enormous trees. The most well-known species of Moringa are *Moringa oleifera* and *Moringa peregrina*, which belong to the family of *Moringaceae*. Saudi Arabia is a major producer of *M. peregrina* [5]. This miracle tree is also known as *M. oleifera* (Mof). Because of its high vitamin, antioxidant, and macronutrient content, leaves are consumed as dietary ingredients to treat nutritional deficiencies [6]. *M. oleifera* leaves offer several beneficial biological properties including antioxidant, anti-atherosclerotic, hypolipidemic, and prevention of heart diseases, making it both nutritional and pharmaceutical important [7] and an important immune activator [8]. Additionally, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Candida albicans*, *C. tropicalis*, and *C. glabrata* are among the microorganisms that are inhibited by *M. peregrine* oil [9]. Aqueous extract of Moringa has been shown to have strong antiproliferative effects on human malignant pancreatic cells [10]. The extraordinary effects of *Moringa oleifera* leaves and bark on colorectal HCT-8 and breast MDA-MB-231 malignant cells have been studied by Al-Asmari, *et al*., which showed that *Moringa oleifera* extracts exhibited cell cycle arrest and apoptosis-mediated cell death in both cell lines, along with alterations in the phenotypic characteristics of the cells [11]. On the other hand, Balamurugan, *et al*. used an MTT experiment to evaluate the anti-tumor effects of Moringa leaf and bark extracts on the HepG2 hepatic cancer cell line. The results demonstrated that the Moringa leaf crude extract had a significantly greater anticancer effect on HepG2 cells than the Moringa bark extract [12]. Additionally, the investigation of the biological substances' effects on various *Moringa oleifera* plant parts has led to the discovery of new action mechanisms, useful advantages, and toxicity profiles. Animal models have, therefore, been employed for toxicological evaluation to show histopathological damage [13]. The goal of the current research was to investigate Saudi moringa oleifera leaf extract's potential preventive and anti-tumor effects against cyclophosphamide-induced male genotoxicity.

Materials and methods

Experimental animals

The animal experimental processes were approved by the Committee of the Research Center, University of Jeddah, Jeddah, Saudi Arabia according to the guidelines and ethical rules. 30 Swiss albino male mice (*Mus musculus*) (MFI strain, 10 - 12 weeks old) from King Fahd Medical Center, King Abdulaziz University, Jeddah, Saudi Arabia were included in this study. The animals were kept in an air-conditioned room (22°C, 45-75% humidity) with a 12 h light/12 h dark cycle.

Preparation of cyclophosphamide and Moringa Oleifera (*Moringa peregrina*) leaves

Endoxan (Baxter Oncology, Halle, Germany), the brand name for cyclophosphamide, was diluted in saline solution and was administrated to the experimental animal intraperitoneally (i.p.) for 7 days straight with the modified mouse dose (6 mg/kg body weight) [14-16].

Moringa Oleifera (*Moringa peregrina*) leaves (MOL) were collected from a private herbarium in the Alula region, Saudi Arabia. The leaves were processed into a fine powder by using a basic hammer mill after being air-dried in a lab for 5 days. The MOL was created by combining 1 g of dried and powdered leaves with 10 mL of boiling water for 5 minutes [10]. The oral administration dose was 200 mg/kg body weight [17].

Animal experiments

The animals were randomly divided into 6 groups with 5 animals per group. Group one (G1) was the control group and was treated with normal saline solution intraperitoneally for 7 days. Group two (G2) was treated with MOL (200 mg/kg) orally for 7 days. Group three (G3) was i.p. treated by therapeutic dose of Endoxan (6 mg/kg) for 7 days. Group four (G4) was co-administration of MOL (oral) and Endoxan (i.p.) at the same time and dosages for 7 days. Group five (G5) was dual-administration with MOL (oral) for 7 days before Endoxan (i.p.) treatment for another 7 days. Group six (G6) was also dual-administration with MOL (oral) for 7 days after 24 h of Endoxan (i.p.) administration for 7 days.

Test for mouse bone marrow chromosomal aberration

Colchicine was intraperitoneally delivered into experimental mice 2 hours before sacrificing animals. 24 hours following the last treatment, animals were sacrificed *via* cervical dislocation. Mouse bone marrow samples were obtained through bone marrow biopsy (aspiration). Cytogenetic analysis was performed by using the method described by Adler [18]. The slides were stained with newly made 2% Giemsa stain the next day for 5 minutes before being rinsed in distilled water to remove any remaining stain. At a magnification of 100x, a total of 50 well-spread metaphase cells from each mouse were scored for chromosomal abnormalities (Figures 1).

Micronucleus (MN) examination

The micronucleus (MN) test was applied to detect chromosomal damage in cells. The presence of micronuclei indicated the improper segregation of chromosomes during cell division. The frequency of micronucleated polychromatic erythrocytes (MNPCEs) was assessed by counting 1,000 polychromatic erythrocytes (PCEs) in each mouse. The criteria used to identify micronuclei were that the cells shared the physical similarities of the typical nuclei, but the nuclei were smaller than that in normal cells (about 1/5 the size of the primary nuclei) [19-21].

Protective effect of MOL

The protective index of MOL against the mutagenic effects of Endoxan on the induction of MN was calculated [22]. The mitotic index and incidence of aberrant cells (in percentages) for each group were analyzed. The percentage of suppressed aberrant cells were calculated according to Shukla and Taneja's method [22].

Statistical analysis

SPSS Statistics Software (22.0) (IBM, Armonk, New York, USA) were employed in this study. The result data were displayed as mean ± standard deviation (SD). ANOVA was used to analyze the differences in total number while calculating the F and P values. The significant difference was defined as $P < 0.05$ (*), while the very significant difference was defined as $P < 0.01$ (**) and the extremely significant difference was defined as $P < 0.001$ (***) [23].

Results

The effects of the treatment with MLE (G2) or Endoxan (G3) alone

After 24 hrs of the last treatment of MOL (200 mg/kg weight), the results showed that there were no significant differences in the total

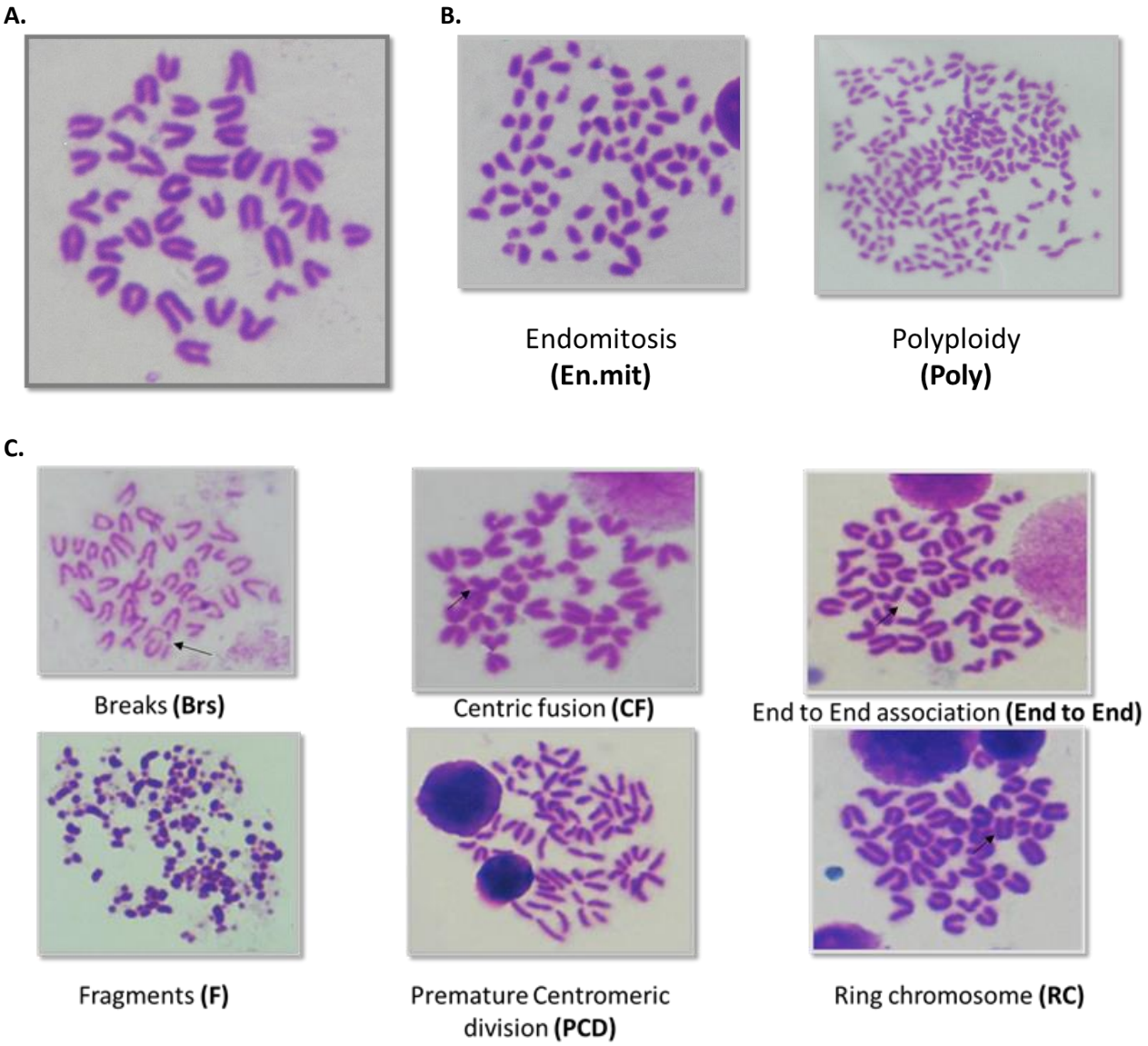

Figure 1. Light micrographs of metaphase spread of mouse bone marrow chromosomes (1,000X). **A.** control group. **B.** Endoxan treated mouse showing numerical chromosomal aberrations. **C.** Endoxan treated mouse showing structural chromosomal aberrations.

Table 1. Effects of MOL, Endoxan, and the dual treatment with MOL and Endoxan on bone marrow chromosomes.

Groups	No. of examined mice	No. of analyzed metaphase	Total St. Abs	Total Num. Abs	Total Ch. Abs
G1	5	250	4 (0.40±0.10)	5 (0.50±0.40)	9 (0.90±0.50)
G2	5	250	10 (1.40±0.50)	6 (0.20±0.37)	14 (1.60±0.63)
G3	5	250	154 (30.00±3.50) ***	30 (5.00±0.77) ***	184 (35.00±4.08) ***
G4	5	250	94 (12.00±3.90) *	25 (1.00±1.00)	119 (13.00±4.48) *
G5	5	250	84 (14.00±4.61) **	18 (2.40±1.17)	102 (16.40±4.60) **
G6	5	250	90 (12.80±4.77) *	32 (0.40±1.25)	122 (12.40±4.99) *

Notes: * $P < 0,05$, ** $P < 0.01$, *** $P < 0.001$. G2 and G3 were compared to G1. G4, G5, G6 were compared to G3.

Table 2. Effects of MOL, Endoxan, and the dual treatment with MOL and Endoxan on bone marrow polychromatic erythrocyte with micronucleus.

Groups	No. of examined mice	No. of cells	No. of PCE/NCE Mean ± SEM	Rate of induction of MN (%)	Rate of antimutagenic effect
G1	5	1,000	22 (0.60±1.20)	0.06	-
G2	5	1,000	19 (0.20±1.11)	0.02	-
G3	5	1,000	237 (43.40±2.92) ***	4.340	-
G4	5	1,000	180 (11.40±3.78) *	1.140	74
G5	5	1,000	95 (28.40±4.86) ***	2.84	34.6
G6	5	1,000	111 (25.20±3.098) ***	2.52	42

Notes: * $P < 0,05$, ** $P < 0.01$, *** $P < 0.001$. G2 and G3 were compared to G1. G4, G5, G6 were compared to G3.

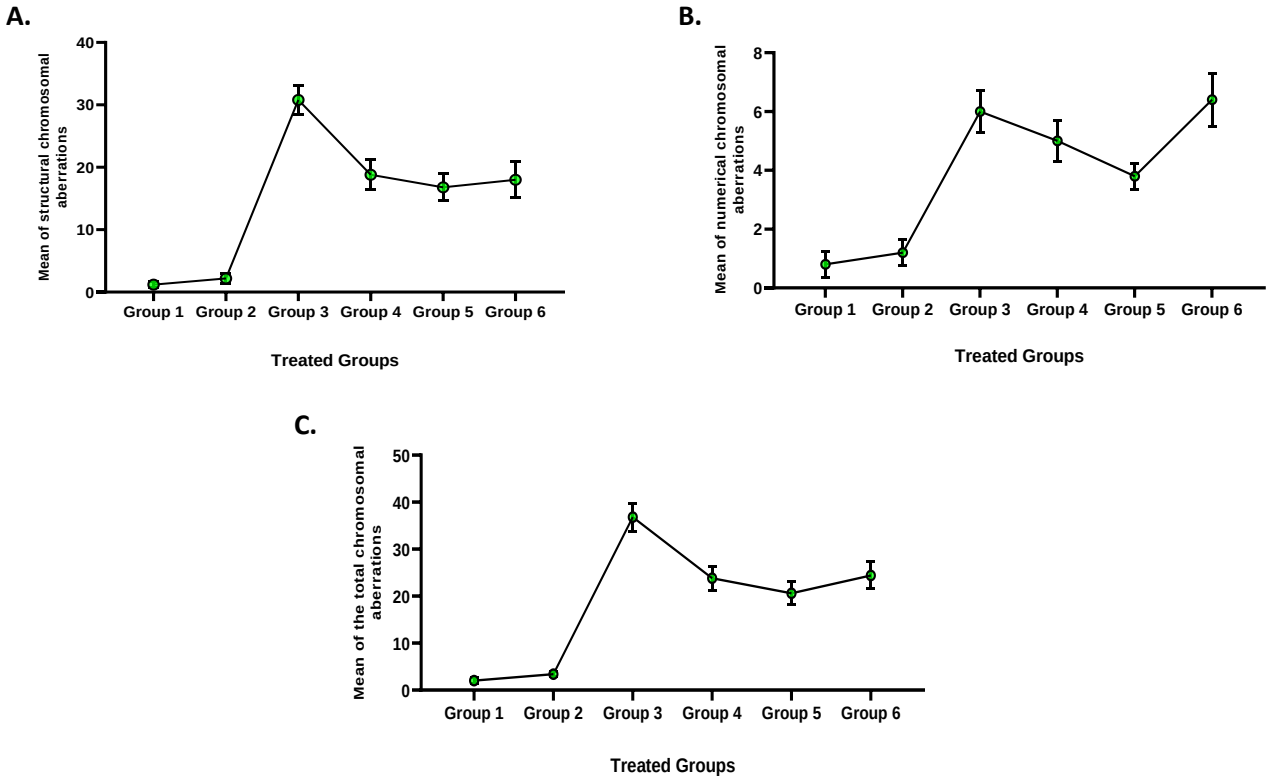

Figure 2. Effects of MOL, Endoxan, and the dual treatment with MOL and Endoxan on the induce of structural chromosomal aberrations (**A**), numerical chromosomal aberrations (**B**), and the total chromosomal aberrations (**C**) of bone marrow in male mice.

number of micronuclei comparing to the control group, while the frequency of bone marrow chromosomal abnormalities did not appear in the current group (G2) (Table 1). On the other hand, the Endoxan alone treatment group (G3) showed the high toxicity on bone marrow cells by extremely significant increase of MNPCEs ($P < 0.001$) to 43.40 ± 2.92 estimated at 4.34%, while the chromosomal aberration increased to 35.00 ± 4.084 including structural and numerical chromosomal aberrations comparing to the control group (G1) (Tables 1 and 2).

The impacts of co- and dual- administration on bone marrow cells

The results showed that Endoxan increased chromosomal aberrations in an extremely significant level (G3) ($P < 0.001$) (Table 1 and Figure 2). However, the treatments with MOL in groups of G4, G5, and G6 showed significant

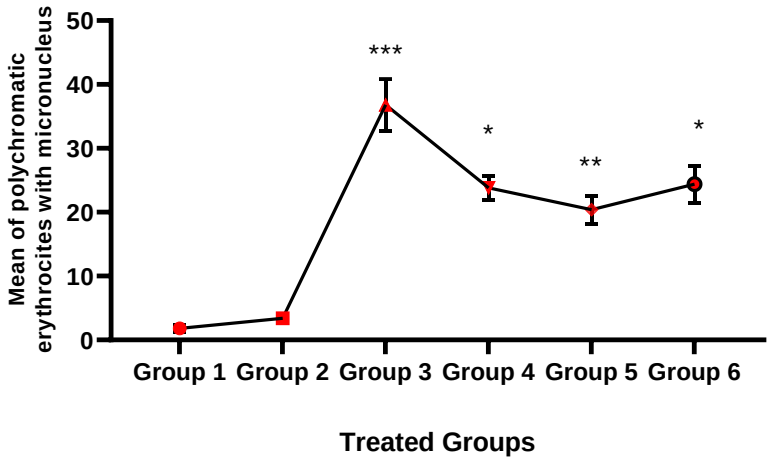

Figure 3. Effects of MLE, Endoxan, and the dual treatment with MLE and Endoxan on the mean of polychromatic erythrocyte with micronucleus of bone marrow in male mice. **P*<0.05, ***P*<0.01, and ****P*<0.001.

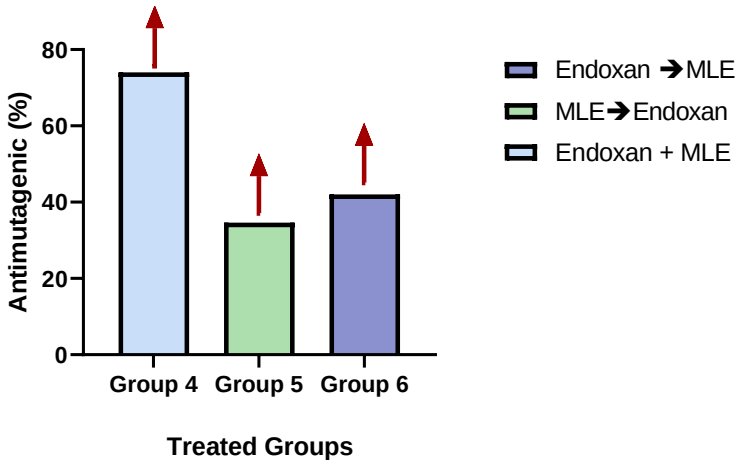

Figure 4. Effect of dual treatment with MLE and Endoxan on the rate of antimutagenic on induce of polychromatic erythrocyte with micronucleus of bone marrow in male mice.

chromosomal aberration (CA) reduction comparing to G3. The mean percentages of the induced CAs were (13.00 ± 4.48, 16.40 ± 4.60, and 12.40 ± 4.99), respectively. In particular, the administration of MOL for 7 days after 24 h of Endoxan i.p. enhanced the ability of bone marrows to reduce the toxicity of Endoxan on the frequency of chromosomal aberrations. Table 2 demonstrated a significant reduction of MNPCEs in G4, G5, and G6 comparing to G3 with the average numbers of 11.40 ± 3.78, 28.40 ± 4.86, and 25.20 ± 3.098, respectively, which were estimated at 1.14, 2.84, and 2.52% reduction rates (Figure 3). The ratio of the anti-mutagenic effects in the inducer of nucleoli showed that precedent, concurrent, and subsequent combined treatments of MOL with Endoxan had produced a noticeable enhancement at 74, 34.6, and 42%, respectively (Figure 4).

Effect of co- and dual- treatments with both MOL and Endoxan on reducing of chromosomal aberrations and micronucleus using analysis of variance and the least significant difference (LSD)

Results of ANOVA analysis demonstrated a highly significant increase in the mean appearance of all chromosomal abnormalities ($P < 0.001$, $F = 38.11$)

between group G3 (Endoxan 6 mg/kg only), co-administration, and dual treatments comparing to the control sample. In addition, the F value of average appearance of micronucleated polychromatic erythrocytes (MNPCEs) was estimated as 32.15. A comparison analysis by using the least significant difference (LSD) showed that dual-administration of MOL for 7 days before Endoxan was the most efficient treatment on reducing the chromosomal aberrations and micronucleus.

Discussion

In the current investigation, the therapeutic dose of Endoxan (6 mg/kg) was administered for 7 days in a row. This was determined by cytogenetic parameters such as the number of micronucleated polychromatic erythrocytes (MNPCEs) and the total number of extremely statistically significant chromosomal abnormalities, which has been supported by previous studies [24-26].

In comparison to the control sample, the results of the micronuclear test demonstrated that Endoxan had a highly significant ability to form MNPCEs in the bone marrow cells. This was in line with the findings from earlier research that used Endoxan to treat bone marrow cells [27, 28]. When spindle fibers of anaphase fail to form, acentric chromatids, chromosomal splinters, or whole chromosomes do not join the nucleus at the end of telophase. These chromosomes are small in comparison to the size of the nucleus and are connected by a nuclear membrane [29]. There are many different types of cancers because of micronuclei. Additionally, after receiving treatment with chemotherapeutic medicines, the micronuclei are used as biological indicators of chromosomal abnormalities, either numerical or structural, and genetic alterations [30-32]. Many statistical methods have been applied for assessing DNA broken, which can be signs of possible mutations [33]. Prior research had shown that Endoxan treatment had resulted in DNA damage and chromosomal abnormalities [34].

The combined treatment of Endoxan and MOL either before or simultaneously injected with showed a significant decrease in the frequency of MNPCEs and the total of chromosomal aberrations comparing to Endoxan alone. According to the resulted data, MOL in Endoxan-MOL co-treatment prevented the rise of MNPCEs and total CAs that Endoxan caused in bone marrow cells by acting as a anticlastogenic and anticytotoxic agent. The combination of Endoxan and MOL did not cause the same degree of MNPCEs and chromosomal abnormalities in the treated animals as it did in the MOL-only or control groups. Because the tested substance was a mixture and multiple ingredients might contribute to the observed impact, an acceptable explanation of this anticlastogenicity or anticytotoxicity was difficult to come across.

According to several academic studies, the current study emphasized on the protective and potential therapeutic effects of MOL against cellular toxicity by lowering oxidants by as free radical scavenging traps, and therefore, eliminated cytotoxicity brought on by chemicals including chemotherapeutic agents. Because MOL was found to limit the generation of micronuclei, the results indicated that MOL prevented the development of mutations as a result of therapy with Endoxan. MOL thus prevented mutations brought on by Endoxan. Breast, pancreatic, and colorectal cancer cells have been shown to grow less rapidly when they exposed to *M. oleifera* leaf and bark extracts [10, 11]. By using gas chromatography-mass spectrometry (GC-MS) analysis, Al-Asmari and colleagues examined 12 distinct chemicals in an *M. oleifera* extract [11] and found that 3 of which might have anticancer effects including isothiocyanates, a powerful anticancer chemical, occurred naturally in an undamaged plant in the form of its precursor, glucosinolates. When the entire plant is disturbed, glucosinolates are said to be hydrolyzed in a reaction facilitated by the enzyme myrosinase to create isothiocyanate

[35]. In addition, it has been demonstrated that phenylmethyl isothiocyanates (PEITC) can slow down the progression of cancer by blocking protein kinase B AKT [36].

Moringa oleifera has the potential to be used as a functional ingredient for human food with its high protein, mineral, dietary fiber, and folate content as well as its low-fat content. It is also a source of Ca, Fe, Cu, and K, all of which have high bioaccessibility, making it a possible source of enriched foods. Additionally, it was shown in this study that *Moringa oleifera* leaves had sufficient levels of phenolic compounds and antioxidant activity to be taken into consideration as a viable source of antioxidant supplements. In general, it might have the ability to help improve the Fe content for nursing moms to combat the issues with Fe deficiency and avoid several illnesses, such as osteoporosis and cardiovascular diseases [37].

The micronuclear test and chromosomal aberration were used in this work to confirm that Endoxan had genotoxic and mutagenic effects that contributed to chromosomal breakage. According to the test results, dual treatment with MOL and Endoxan significantly reduced the cytotoxic and genotoxic effects of Endoxan. According to the results of the current investigation, MOL's capacity to reduce Endoxan's cytotoxic and genotoxic effects might be explained by the substance's strong antioxidant properties.

References

1. Valko V, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. 2007. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 39(1):44–84.
2. Silverman RB, Holladay MW: From The Organic Chemistry of Drug Design and Drug Action. 3rd Edition. Elsevier Inc. 2015.
3. Fauzi AN, Norazmi MN, Yaacob NS. 2011. Tualang honey induces apoptosis and disrupts the mitochondrial membrane potential of human breast and cervical cancer cell lines. Food Chem Toxicol. 49(4):871-878.
4. Afkhami-Ardakani M, Hasanzadeh S, Shahrooz R, Delirezh N, Malekinejad H. 2021. Spirulina platensis (*Arthrospira platensis*) attenuates cyclophosphamide-induced reproductive toxicity in male Wistar rats: evidence for sperm apoptosis and p53/Bcl-2 expression. J Food Biochem. 2021:e13854.
5. Alaklabi. 2015. Genetic diversity of *Moringa peregrina* species in Saudi Arabia with ITS sequences. Saudi J Biol Sci. 22(2):186–190.
6. Asare GA, Gyan B, Bugyei K, Adjei S, Mahama R, Addo P, *et al*. 2012. Toxicity potentials of the nutraceutical *Moringa oleifera* at supra-supplementation levels. J Ethnopharmacol. 139(1):265–272.
7. Mabrouki L, Rjeibi I, Taleb J, Zourgui L. 2020. Cardiac ameliorative effect of *Moringa oleifera* leaf extract in high-fat diet-induced obesity in rat model. Biomed Res Int. 2020:6583603.
8. Monir W, Abdel-Rahman MA, Hassan SE, Awad SM. 2020. Pomegranate peel and moringa-based diets enhanced biochemical and immune parameters of Nile tilapia against bacterial infection by *Aeromonas hydrophila*. Microb Pathogen. 145:104202.
9. Lalas SI, Tsaknis J. 2002. Extraction and identification of natural antioxidant from the seeds of the *Moringa oleifera* tree variety of Malawi. J Amer Oil Chem Soc. 79:677-683.
10. Berkovich L, Earon G, Ron I, Rimmon A, Vexler A, Lev-Ari S. 2013. *Moringa oleifera* aqueous leaf extract down-regulates nuclear factor-kappaB and increases cytotoxic effect of chemotherapy in pancreatic cancer cells. BMC Complem Alt Med. 13:1-7.
11. Al-Asmari AK, Albalawi SM, Athar MT, Khan AQ, Al-Shahrani H, Islam M. 2015. *Moringa oleifera* is an anti-cancer agent against breast and colorectal cancer cell lines. PloS One. 10(8):e0135814.
12. Balamurugan V, Balakrishnan V, Robinson JP, Ramakrishnan M. 2014. Anticancer and apoptosis-inducing effects of *Moringa concanensis* using hepG2 cell lines. Bangladesh J Pharm. 9(4):604-609.
13. Nikkon F, Habib MR, Saud ZA, Karim MR, Roy AK, Zaman S. 2009. Toxicological evaluation of chloroform fraction of flower of *Tagetes Erecta* L. on rats. Int J Drug Dev Des. 1(1):161-165.
14. Kwon Y, Lee KW, Park H, Son JK, Lee J, Cho CW, *et al*. 2019. Cyclophosphamide and fludarabine monophosphate dose optimization for the non-myeloablative condition in non-human primates to induce transient mixed chimerism via bone marrow transplantation. American J Trans Res. 11(10):6444–6453.
15. Anton E. 1997. Ultrastructural changes of stromal cells of bone marrow and liver after cyclophosphamide treatment in mice. Tissue Cell. 29(1):1–9.
16. Paget GE, Barnes JM: From Toxicity Test. In: Evaluation of Drug Activities. Volume 6. Edited by Laurence, D.R Bacharach, A.L. Massachusetts: Academic Press. 1964:135-166.
17. Adedapo A, Falayi O, Oyagbemi H. 2015. Evaluation of the analgesic, anti-inflammatory, antioxidant, phytochemical and toxicological properties of the methanolic leaf extract of commercially processed Moringa oleifera in some laboratory animals. J Basic Cli. 26(5):491-499.
18. Adler SS. 1984. Hemopoietic reconstitution of marrow and spleens in mice after whole-body irradiation and marrow transplantation. Transplantation. 37(5):517-520.

19. Schmid W. 1975. The Micronucleus Test. Mutation Res. 31:9-15.
20. Hayashi M, Sofuni T, Ishidate M. 1984. Kinetics of micronucleus formation in relation to chromosomal aberrations in mouse bone marrow. Mutation Res. 127(2):129–137.
21. Albanese R, Middleton BJ. 1987. The assessment of micronucleated polychromatic erythrocytes in rat bone marrow. Technical and statistical considerations. Mutation Res. 182(6):323–332.
22. Shukla Y, Taneja P. 2002. Antimutagenic effects of garlic extract on chromosomal aberrations. Cancer Lett. 176(1):31–36.
23. Haseman JK, Soares ER. 1976. The distribution of fetal death in control mice and its implications on statistical tests for dominant lethal effects. Mutation Res. 41(2-3):277–288.
24. Liu H, Feng X, Wu S, Zong T, Li B, Zhang Z. 2019. Vitamin D resists cyclophosphamide-induced genomic and dna damage in chl cells *in vitro* and in mice *in vivo*. Nutr Cancer. 71(6):1030–1039.
25. Tripathi R, Banji D, Tripathi P. 2022. Evaluation of the mutagenic and anti-mutagenic potential of alpha-lipoic acid by chromosomal aberration assay in mice. Drug Chem Tox. 43(4):378–382.
26. Atlı Şekeroğlu Z, Gediz Ertürk A, Kontaş Yedier S, Şekeroğlu V. 2021. *In vitro* cytogenetic activity of 3-amino-4-[4-(dimethylamino) phenyl]-4, 5-dihydro-1, 2, 5-thiadiazole 1, 1-dioxide. Drug Chem Toxicol. 44(6):595-600.
27. Kishino Y, Hasegawa T, Kato A, Nishiya Y, Rozhnal V, Watanabe K, *et al*. 2019. Effect of inter-individual variability in human liver cytochrome P450 isozymes on cyclophosphamide-induced micronucleus formation. Mutation Res/Gen Toxic En Mutagen. 838:37–45.
28. Kishino Y, Hasegawa T, Yamoto T, Mori K. 2019. Species differences in micronucleus induction of the clastogenic compounds associated with drug metabolic profile. J Toxic Sci. 44(10):701–709.
29. Fenech M, Kirsch-Volders M, Natarajan AT, Surralles J, Crott JW, Parry J, *et al*. 2011. Molecular mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud formation in mammalian and human cells. Mutagenesis. 26(1):125–132.
30. Grover IS, Kaur S. 1999. Genotoxicity of wastewater samples from sewage and industrial effluent detected by the Allium root anaphase aberration and micronucleus assays. Mutation Res. 426(2):183–188.
31. Krishna G, Hayashi M. 2000. *In vivo* rodent micronucleus assay: protocol, conduct and data interpretation. Mutation Res. 455(1-2):155–166.
32. Bakare AA, Okunola AA, Adetunji OA, Jenmi HB. 2009. Genotoxicity assessment of a pharmaceutical effluent using four bioassays. Genetics Mol Bio. 32(2):373–381.
33. Araldi RP, de Melo TC, Mendes TB, de Sá Júnior PL, Nozima BH, Ito ET, *et al*. 2015. Using the comet and micronucleus assays for genotoxicity studies: A review. Biomed Pharmac. 72:74–82.
34. Gamal-Eldeen AM, Abo-Zeid MA, Ahmed EF. 2013. Anti-genotoxic effect of the Sargassum dentifolium extracts: Prevention of chromosomal aberrations, micronuclei, and DNA fragmentation. Exp Tox Pathol. 65(1-2):27-34.
35. Fahey JW, Zalcmann AT, Talalay P. 2001. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochem. 56(1):5–51.
36. Gao N, Budhraja A, Cheng S, Liu EH, Chen J, Yang Z, *et al*. 2011. Phenethyl isothiocyanate exhibits antileukemic activity *in vitro* and *in vivo* by inactivation of Akt and activation of JNK pathways. Cell Death Dis. 2(4):e140.
37. Peñalver R, Martínez-Zamora L, Lorenzo JM, Ros G, Nieto G. 2022. Nutritional and antioxidant properties of moringa oleifera leaves in functional foods. Foods. 11(8):1107.