Genetic Protection with Portulaca oleracea: Cytogenetic Analysis of Dose-Dependent Herbal Response to Mutagenic Damage

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Research Article

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Genetic Protection with *Portulaca oleracea*: Cytogenetic Analysis of Dose-Dependent Herbal Response to Mutagenic Damage

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Abstract:

Background: In this study, the antigenotoxic effects of ethanol (PO_{eth}) and chloroform (PO_{chl}) extracts of *Portulaca oleracea* (purslane) against DNA damage induced by common genotoxic agents, ethyl methane sulfonate (EMS), mitomycin-C (MMC) and N-nitroso-N-ethylurea (ENU) were evaluated. Genomic damage and cytotoxic effects were analyzed by Sister Chromatid Exchange (SCE) and Micronucleus (MN) tests applied to human peripheral lymphocytes.

Results: Statistically significant increases in SCE and MN frequencies were detected in groups treated with EMS, MMC and ENU ($P<0.05$). In contrast, no significant genotoxicity was observed in groups in which PO_{eth} and PO_{chl} extracts were applied alone. Importantly, the use of these extracts together with genotoxic agents provided dose-dependent significant decreases in damage markers. The most significant protective effect was observed at 4 ppm extract doses, and significant decreases were recorded in SCE and MN rates.

Conclusions: This effect is thought to be due to the capacity of antioxidant compounds such as flavonoids, betalains, and ascorbic acid contained in *P. oleracea* to neutralize reactive oxygen species and possibly support DNA repair mechanisms. The findings show that *P. oleracea* has the potential to protect genome integrity under genotoxic stress and contribute to the development of plant-based

protective strategies. The obtained findings are promising that dietary intake of purslane or its pharmacological formulations can be used to prevent DNA damage in case of exposure to genotoxic chemicals.

Keywords: Sister Chromatid Exchange (SCE), Micronucleus (MN), *Portulaca oleracea*, antigenotoxic effect, genotoxicity, ethanol extract, chloroform extract.

Background

All organisms are constantly exposed to DNA damaging agents in natural living conditions. DNA damage occurs by endogenous cellular metabolic processes such as hydrolysis, oxidation, alkylation, and DNA base mismatches, or by exogenous sources such as ultraviolet, ionizing radiation, and chemicals. Most cancers are caused by exposure to external chemicals. Damage caused by agents to DNA in the cell is the beginning of the carcinogenesis process. DNA repair processes work non-stop to repair damage. However, replication of damaged DNA may also occur prior to repair. This leads to gene mutations and the formation of altered proteins. The complex process of carcinogenesis includes many such events (1). Damaged DNA that is not repaired properly can lead to genomic instability leading to tumorigenesis. To maintain genomic stability, mammalian cells have evolved DNA repair mechanisms (2). DNA repair mechanisms eliminate or treat damage to ensure survival. At this stage, disruption or dysregulation of DNA repair leads to genome instability. It destabilizes cellular metabolic homeostasis (3).

Genetic toxicology is a branch of science that studies the damage caused to DNA by chemicals, drugs, and environmental agents and the changes in genetic material. This field is critical for understanding the mechanisms of mutations, chromosomal aberrations, and carcinogenic processes (4). Genetic toxicity tests are widely used in the safety assessment of chemicals, drug development processes, and monitoring industrial exposures to protect human health and ecosystems (5). A genotoxic agent (or

genetic toxicant) refers to a substance that causes heritable changes in the genetic markers of an organism. These agents affect the gametes in the gonads and lead to a decrease in the number of gametes or changes in genetic information. When gametes from two different sexes fuse, the resulting zygote, if it survives, may be genetically different from its parents. Such permanent changes in genotype are called "mutations" and the agents that cause them are called "mutagens". Mutations can also occur in somatic cells, but they are not hereditary. However, such changes are particularly important because they can contribute to carcinogenesis (6,7).

Commonly used positive controls in genotoxicity testing include ethyl methanesulfonate (EMS), mitomycin-C (MMC), and N-Nitroso-N-ethylurea (ENU), which are known for their mutagenic properties (8). These agents differ significantly in their genotoxicity mechanisms.

EMS is a mutagenic, teratogenic, and carcinogenic organic compound and an alkylating agent that has been used to study mutagenesis for more than 50 years. It transfers ethyl groups to DNA bases, causing point mutations via replication errors. Mismatching leads to transition mutations (e.g. GC → AT) and frameshift mutations (9). EMS alkylates the phosphate groups in the DNA backbone, causing chain breaks and apurinic regions (10). It produces alkyl adducts that disrupt base pairing by primarily targeting guanine at positions O^6 and N^7 (11).

MMC, a bioreductive alkylating agent, forms DNA interstrand crosslinks (ICL's). These cross-links block replication and transcription, leading to mutations or cell death. ICL's physically block replication forks, causing collapse and double-strand breaks (12). MMC also contributes to oxidative damage by generating reactive oxygen species (ROS) via redox cycling (13,14). Insoluble ICL's trigger chromatid exchanges and ROS-mediated oxidative stress (12,15). Although MMC is a potent anticancer drug, its mutagenic and carcinogenic properties pose a risk for secondary malignancies (16).

ENU, a potent nitrosamine derivative, forms O^6-ethylguanine in DNA, leading to G:C→A:T transitions. It also induces other base substitutions (AT→GC, AT→CG, AT→TA, GC→CG, GC→TA) (17). ENU exposure leads to high mutation frequencies, genetic instability, DNA strand

breaks, and oxidative damage (18,19). Its mutagenicity is associated with alkylation of oxygen or nitrogen atoms in DNA bases (20,21).

Increasing concerns about genotoxic agents have stimulated research on natural compounds with protective effects. Genotoxicity testing not only identifies harmful agents but also evaluates antigenotoxic substances for therapeutic use. Historically, natural materials, especially plants, have been an integral part of human health. Ancient practices such as herbal teas, poultices, and pastes continue today to treat diseases and combat toxins. Linking traditional and modern medicine, pharmacognosy focuses on deriving bioactive compounds from plants for drug development. Phytotherapy or herbal medicine remains relevant in the treatment of ailments, including cancer, despite advances in medical science. Approximately 25% of prescription drugs are of plant origin (22) and there are 650 medicinal plant species in Türkiye's flora (23).

Plant extracts are increasingly investigated for their ability to reduce damage caused by genotoxic agents. Particularly rare plant species contain compounds that suppress mutagenic and carcinogenic effects (24). DNA damage and oxidative stress form the basis of pathologies such as cancer and aging (25). Natural plant compounds show protective activity against genotoxicity caused by oxidative stress (7,26,27). Fruits and vegetables rich in phytochemicals show antimicrobial, antioxidant, kızılantimutagenic and anticarcinogenic properties (28–30).

P. oleracea, widely used, recognized as a medicinal plant and designated as a "global medicinal plant" by the World Health Organization (31,32), is rich in antioxidants, anti-inflammatory agents and anticancer compounds (27). Historically used for its therapeutic properties, purslane is also valued as a traditional kitchen ingredient. It has the highest omega-3 fatty acid content among green leafy vegetables (33,34) and contains vitamins (A, B1, B2, C, niacinamide, nicotinic acid, α-tocopherol, β-carotene), minerals (magnesium, calcium, potassium, iron) and two betalain alkaloid pigments (red betacyanins and yellow betaxanthins) that have been shown to have antioxidant and antimutagenic effects (35).

104 This study evaluates the protective effects of ethanol (PO_{eth}) and chloroform (PO_{chl}) extracts of
105 Portulaca oleracea (purslane) against genotoxicity induced by EMS, MMC and ENU. In this context,
106 sister chromatid exchange (SCE) test and micronucleus (MN) test are two main methods to determine
107 genomic and oxidative damage in lymphocyte cells.
108 The SCE test analyzes the exchange of genetic material between non-homologous chromatids during
109 cell division. In this test, DNA replication is monitored by adding bromodeoxyuridine (BrdU) to cell
110 culture. SCE's, visualized by fluorescence or Giemsa staining techniques, are an indicator of exposure
111 to genotoxic agents, particularly DNA cross-linking agents or free radicals (36). High SCE frequency
112 indicates activation of DNA repair mechanisms, thus playing an important role in cancer research and
113 drug toxicity studies (37).
114 The micronucleus test detects small nuclei (micronuclei) formed by chromosome fragments or
115 complete chromosomes that have separated from the main nucleus during cell division. This test can
116 be performed in in vitro (cell culture) or in vivo (human lymphocytes) models (38). Using
117 cytokinesis-blocked cells, clastogenic (chromosome breakage) and anegenic (chromosome loss)
118 effects can be assessed simultaneously. The micronucleus test has been standardized by OECD (No.
119 487) and ICH guidelines (39) and is used in a wide range of areas from the pharmaceutical industry
120 to environmental monitoring (40).
121 Both tests are complementary to each other in determining genotoxic risks. The SCE test targets
122 damage at the DNA level, while the MN test targets anomalies at the chromosomal level. These
123 methods are an indispensable part of toxicological risk assessments and guide strategies to protect
124 human and environmental health (41).
125
126 **Methods**
127
128 **Chemicals**

Mitomycin-C (MMC) (cat. No: M5353), Ethyl methanesulfonate (EMS) (M0880), N-Nitroso-N-ethylurea (ENU) (Cas no. 759-73-9), methanol (CAS No:67-56-1), dimethyl sulfoxide (CAS No:6768-5), 5–bromo–2–deoxyuridine (CAS No: 59-14-3), potassium chloride (CAS No: 7447-40-7), giemsa (CAS No: 51811-82-6), bisbenzimide H 33342 (CAS No:23491-52-3), sodium citrate (CAS No: 613204-3), sodium chloride (CAS No:7647-14-5), acetic acid (CAS No: 64-19-7),chromosome medium (CAS No: F 5023), colchicine (CAS No: L 6221) and cytochalasin-B (CAS No: 14930-96-2) were purchased from the Sigma-Aldrich Company.

Preparation of the methanol extracts of *Portulaca oleracea* L.

Purslane plant, which was determined to be used as an antigenotoxic agent in the experiments, was collected from the vicinity of Hasancık village in Adıyaman province and from an altitude of 600-900 meters. The area where the samples were collected is public land belonging to the Non-Timber Forest Products and Services Department of the General Directorate of Forestry, Ministry of Forestry and Water Affairs of the Republic of Turkey, and is not subject to any permit (CIRCULAR NO: 302 INVENTORY AND PLANNING OF NON-TIMBER FOREST PRODUCTS AND PRINCIPLES OF PRODUCTION AND SALE). Taxonomic identification was conducted by Prof. Dr. Özkan Eren (Aydın Adnan Menderes University, Faculty of Science, Department of Biology, Division of Botany). A voucher specimen (AYDN 12317) was deposited in the Herbarium of Adnan Menderes University (AYDN), Aydın, Türkiye. Ethanol and chloroform extract (PO_{eth}, PO_{chl}) were prepared with all of the above-ground organs of the purslane plant, such as stem, leaves and flowers, which were collected in its natural environment, during the flowering period and from lands far from agriculture (29). PO_{eth} and PO_{chl} extract were dissolved with DMSO in the course of applications.

Donors for peripheral blood assays

All donors were determined according to Kızılet *et al*., (7). This study complies with all provisions of the Declaration of Helsinki. Informed consent was obtained from all participants in the study. It was approved by the Aydın Adnan Menderes University Faculty of Medicine, Non-Interventional Clinical Research Ethics Committee (DECISION:02 Protocol No: 2025/120).

Sister chromatid exchange (SCE) assay

To determine the genotoxic and antigenotoxic effects of EMS, MMC and ENU (1 ppm) and different concentrations PO_{eth} and PO_{chl} (1, 2 and 4 ppm) by SCE, 1–2.5 mL of human peripheral blood was added to 5 mL chromosome media. Different sets were prepared for each concentration and each donor. 5–bromo–2–deoxyuridine (BrdU) was added to all experiment sets at a final concentration of 10^{-4}M. All substances added to the tubes were sterilized to prevent contamination during the experimental stage. The experimental sets were incubated for 72 hours at 37 °C in dark incubators. At 70 hours of the experiment, colchicine was added to the medium at a final concentration of 0.5 µg/mL to stop the mitosis at the metaphase stage. At the end of the 72 hours incubation, the tubes were centrifuged and removed the supernatants. Hypotonic solution (0.075 M KCl) was added onto pellet and incubated for 30 minutes in dark incubator at 37 °C. At the end of the time, the supernatant was removed after centrifugation. The remaining pellet was washed with a fixative consisting of a methanol/acetic acid (3:1 v/v) mixture. This procedure was repeated three times. Peripheral blood smears were then prepared from the remaining pellet and allowed to dry in the dark for 3 days. These preparations were stained according to Czepulkowski and Rooney (42) fluorescence plus Giemsa method. For this purpose, preparations were wetted with 0.5 µg/mL bisBenzimide (Hoechst 33342) for 20 minutes. Wet preparations were incubated for 1 hour under 366 nm UV. The preparations were then kept in a 2 X SSC (1:1 v/v 0.03 M sodium citrate/0.03 M sodium chloride) solution in a 65 °C water bath for 1 hour. Finally, the preparations were stained with 5% giemsa. Preparations were examined at 1000 X magnification and sister chromatid changes were recorded on chromosomes in metaphases. In addition, the replication index (RI) was calculated to determine the effects on DNA

replication. For this calculation, 100 randomly selected metaphase plates were separated from the first, second and third metaphase stages, counted, recorded and calculated.

Micronucleus assays

EMS, MMC and ENU (1 ppm) and different concentrations of PO_{eth} and PO_{chl} (1, 2 and 4 ppm) were used to determine the MN ratio in human peripheral lymphocytes using the micronucleus test developed by Fenech (38) and Kirsch-Volders *et al.*, (43) was modified and used (7). 0.25 ml of blood sample taken from the donor was added to 5 ml of prepared medium (chromosome medium b). It was immediately incubated at 37±1 ° C. At 24 hours, 1 ppm EMS, 1 ppm MMC, 1 ppm ENU and different concentrations of extract (1, 2 and 4 ppm) were added to EMS+PO_{eta}, EMS+PO_{chl}, MMC+PO_{eta}, MMC +PO_{chl} and ENU+PO_{eta}, ENU+PO_{chl} tubes separately. In addition to these, distilled water and 1% dimethyl sulfoxide (DMSO) as a solvent were prepared as negative controls. At the 48th hour, cytochalasin-B was added to each tube at a final concentration of 3 µg/mL to prevent cytokinesis. At the 72nd hour, the tubes were centrifuged at 1000 rpm and the supernatant was discarded, and hypotonic solution was added instead. The tubes, which were kept at 37±1°C for five minutes, were centrifuged again and the pellet part was fixed with the fixation solution. After the last centrifugation process, smear preparations were prepared from the remaining pellet and stained with the giemsa staining technique. These preparations were examined under a light microscope at 10x100 magnification, and MN counting was performed in binucleate cells (BNH) according to the criteria determined by Countryman and Heddle (44). In addition, in order to determine the cytotoxic effect of each pesticide, a thousand cells were randomly counted and the nuclear division index (NDI) was calculated according to the number of nuclei.

Statistical methods

The sample size to be used in the SCE and MN tests was determined to be consistent with similar studies in the literature and to ensure statistical significance (27,45,46). The sample size for both tests

was calculated using the G*Power 3.1 statistical analysis program with a 95% confidence level (α = 0.05) and 80% test power ($1-\beta = 0.80$) based on previously reported effect sizes.

For the SCE test, the method reported by Latt *et al.*, (47) and Perry and Wolff (36) was used; a total of 100 metaphase plates, 25 from each donor, and a total of 400 metaphase plates will be analyzed for replication index (RI) evaluations. This sample size is at a level that will provide statistical significance in detecting a 20% increase (48).

For the MN test, the protocol suggested by Fenech (38) and used in human peripheral lymphocytes was taken into account; 1000 binucleated cells will be evaluated in each application group. This number of cells was considered sufficient to detect 30% changes in micronucleus frequency (38).

The determined sample size aimed to keep the coefficient of variation (%CV) of the tests low and to measure biological effects reliably.

In the study, the effect size was calculated based on average differences and standard deviations (49–51).

A total of 100 metaphase plates, 25 from each donor, were analyzed for the SCE test, and a total of 400 metaphase plates, 100 from each donor, were analyzed for the Replication Index (RI). This design was structured to allow the determination of 15–25% differences in chromosomal rearrangements, in accordance with the standards recommended by Latt *et al*., (47) and Perry and Wolff (36).

For the MN test, 1000 binucleated cells were examined in each treatment group. This sample size was considered sufficient to reliably detect 20–30% changes in micronucleus frequency, in accordance with the minimum cell number criteria reported by Fenech (38) and Kirsch-Volders *et al*., (43).

SPSS (Statistical Package for Social Sciences) version 22.0 software (IBM Corp., Armonk, NY, USA) was used for data analysis in this study. Categorical data of the groups were compared using the chi-square test. Continuous variable data were tested for normality using the Shapiro-Wilks test and homogeneous distribution was tested using the Levene test. Continuous variable data showing normal distribution were compared using the independent sample t-test. Data showing non-normal

distribution were compared using the Mann-Whitney U test. P values less than 0.05 were considered statistically significant.

Results

SCE findings for control and purslane extracts

For this purpose, two separate control groups were created. The negative control group was distilled water and DMSO, which was the solvent of all application groups. The average SCE's for distilled water and DMSO were 3.59±0.05, 3.69±0.07 (Table 1), respectively, and the difference between them was statistically insignificant (P>0.05). For this reason, the application groups were compared with DMSO. Only the data obtained from the application of purslane ethanol (PO_{eth}) and chloroform (PO_{chl}) extracts were 3.78±0.09, 3.82±0.05, respectively (Figure a) (P>0.05).

In addition, the replication index (RI) was calculated for each application. The calculated RI values for distilled water, DMSO, PO_{eth} and PO_{chl} are 2.41±0.04, 2.25±0.06, 2.38±0.06 and 2.36±0.03, respectively (Table 1). According to these results, the difference between the control groups for RI values was found to be statistically insignificant (P>0.05).

Table 1. SCE, RI values and statistical analysis results of the control and purslane groups

Application groups	1st donor	2nd donor	3rd donor	SCE/Cell (Average)	Min.-Max. SCE	M1	M2	M3	RI
Distilled water	3,64	3,55	3,57	3,59±0,05	1-11	45	87	168	2,41±0,04
DMSO (%1)	3,76	3,60	3,72	3,69±0,07	1-10	46	95	146	2,25±0,07
PO_{eth} (1ppm)	3,78	3,76	3,80	3,78±0,09	1-10	47	96	158	2,38±0,06
PO_{chl} (1ppm)	3,80	3,82	3,84	3,82±0,05	1-11	45	91	160	2,36±0,03

Min.=minimum, Max.=maximum, [a]Significant at 0.05 level compared to DMSO, P=0.05

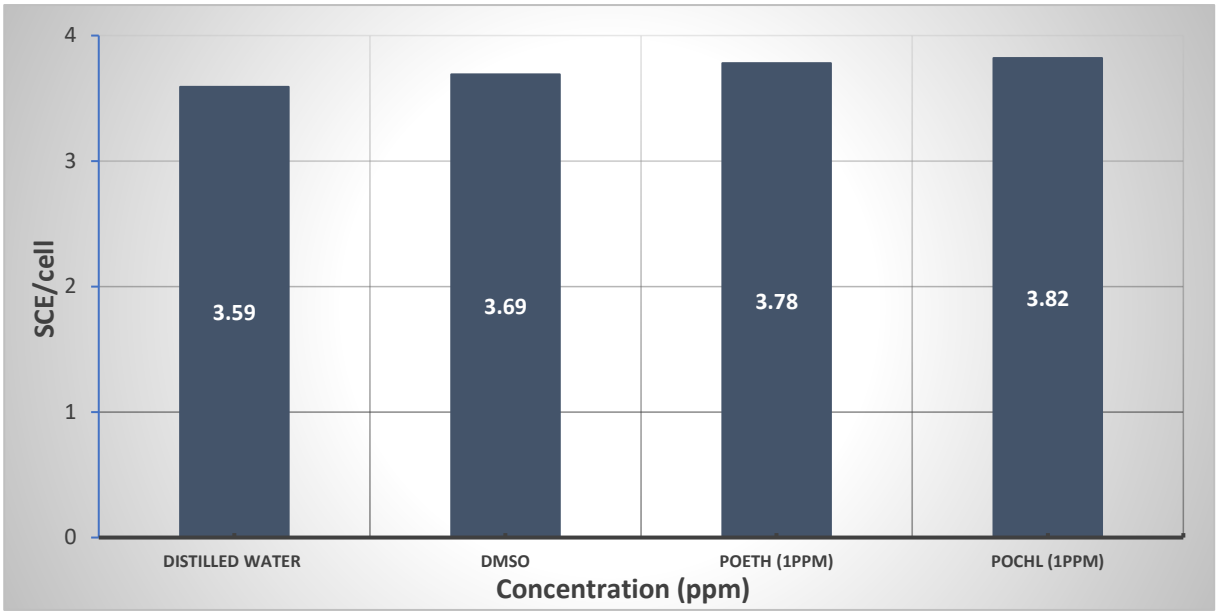

Figure a. SCE/Cell data of control groups

SCE findings of EMS, MMC and ENU

In the study, the average SCE's for 1 ppm EMS, 1 ppm MMC and 1 ppm ENU were 22.31±0.03, 27.18±0.05 and 21.16±0.04, respectively (Figure b), and were found to be significant at the $P<0.05$ level when compared with DMSO (Table 2). In all applications, the highest SCE's in metaphase plates that had undergone the second mitosis were 42, 45 and 40 SCE/Cell, respectively.

RI values were measured as 2.03±0.07, 2.01±0.01 and 2.05±0.03 for EMS, MMC and ENU, respectively (Table 2). According to the findings, EMS, MMC and ENU reduced replication. However, it is statistically insignificant ($P>0.05$).

Table 2. SCE, RI values and statistical analysis results of EMS, MMC and ENU application groups

Application groups	1st donor	2nd donor	3rd donor	SCE/Cell (Average)	Min.-Max. SCE	M1	M2	M3	RI
Distilled water	3,64	3,55	3,57	3,59±0,05	1-11	45	87	168	2,41±0,04
DMSO (%1)	3,76	3,60	3,72	3,69±0,07	1-10	46	95	146	2,25±0,07
EMS (1ppm)	21,21	23,95	21,76	22,31±0,03[a]	7-42	45	84	132	2,03±0,07
MMC (1ppm)	28,67	27,82	25,05	27,18±0,05[a]	11-45	45	87	128	2,01±0,01
ENU (1ppm)	21,01	22,32	20,15	21,16±0,04[a]	5-40	46	90	130	2,05±0,03
Min.=minimum, Max.=maximum, [a]Significant at 0.05 level compared to DMSO, P=0.05									

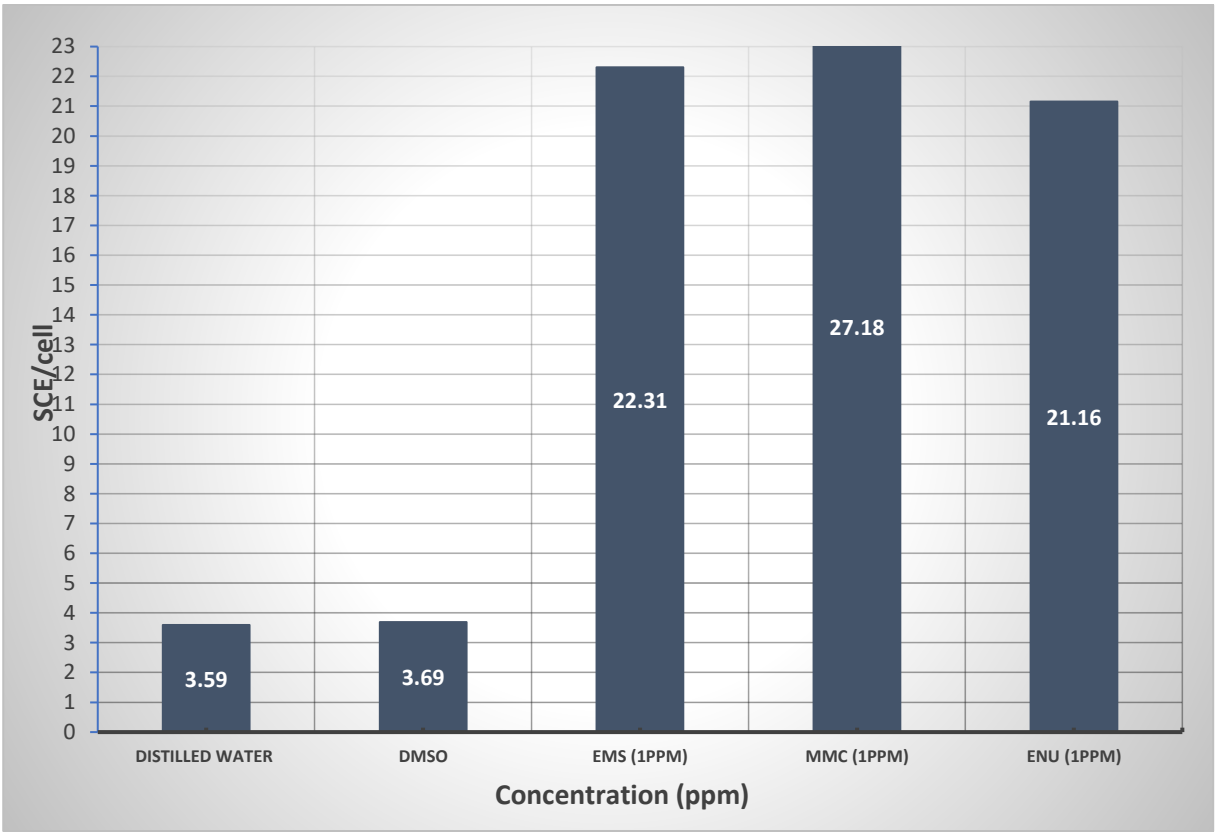

Figure b. SCE/Cell data of EMS, MMC and ENU application groups

SCE findings obtained as a result of PO_{eth} and PO_{chl} application against EMS

To observe the effects of EMS+PO_{eth} and EMS+PO_{chl} on SCE, 1 ppm EMS was applied to blood cultures at twice and four times the concentration (1:1/v:v, 1:2/v:2v, 1:4/v:4v). The obtained SCE frequencies and RI values are presented in Table 3.

The observed SCE/Cell ratios were 12.13±0.04, 14.16±0.07, 7.07±0.03, 7.11±0.03, 3.87±0.05 and 3.88±0.01 for 1EMS+1PO_{eth}, 1EMS+1PO_{chl}, 1EMS+2PO_{eth}, 1EMS+2PO_{chl}, 1EMS+4PO_{eth} and 1EMS+4PO_{chl}, respectively (Table 3). The decrease in SCE/Cell ratios was significant for antigenotoxicity ($P<0.05$) (Figure c).

For the antigenotoxicity study, RI was also calculated in the application groups. RI values for 1EMS+1PO_{eth}, 1EMS+1PO_{chl}, 1EMS+2PO_{eth}, 1EMS+2PO_{chl}, 1EMS+4PO_{eth} and 1EMS+4PO_{chl} were 2.55±0.01, 2.49±0.05, 2.50±0.04, 2.50±0.02, 2.54±0.01 and 2.56±0.03, respectively (Table 3). Although RI values increased compared to the DMSO control group, the results were statistically insignificant at $P>0.05$.

Table 3. SCE, RI values and statistical analysis results of EMS, PO_{eth} and PO_{chl} application groups

Application groups	1st donor	2nd donor	3rd donor	SCE/Cell (Average)	Min.-Max. SCE	M1	M2	M3	RI
Distilled water	3,64	3,55	3,57	3,59±0,05	1-11	45	87	168	2,41±0,04
DMSO (%1)	3,76	3,60	3,72	3,69±0,07	1-10	46	95	146	2,25±0,07
EMS (1ppm)	21,21	23,95	21,76	22,31±0,03[a]	7-42	45	84	132	2,03±0,07
1EMS+1PO_{eth}	14,10	10,16	12,14	12,13±0,04[b]	5-25	43	97	176	2,55±0,01
1EMS+1PO_{chl}	14,12	14,21	14,15	14,16±0,07[b]	5-25	44	95	171	2,49±0,05
1EMS+2PO_{eth}	7,04	7,07	7,11	7,07±0,03[b]	4-13	45	90	175	2,50±0,04
1EMS+2PO_{chl}	7,13	7,10	7,11	7,11±0,03[b]	3-17	45	88	176	2,50±0,02
1EMS+4PO_{eth}	3,84	3,92	3,86	3,87±0,05[b]	1-12	46	88	180	2,54±0,01

1EMS+4PO$_{chl}$	3,86	3,86	3,92	3,88±0,01[b]	1-11	46	89	181	2,56±0,03

Min.=minimum, Max.=maximum, [a]=Significant at 0.05 level compared to DMSO, [b]=Significant at 0.05 level according to EMS, P=0,05

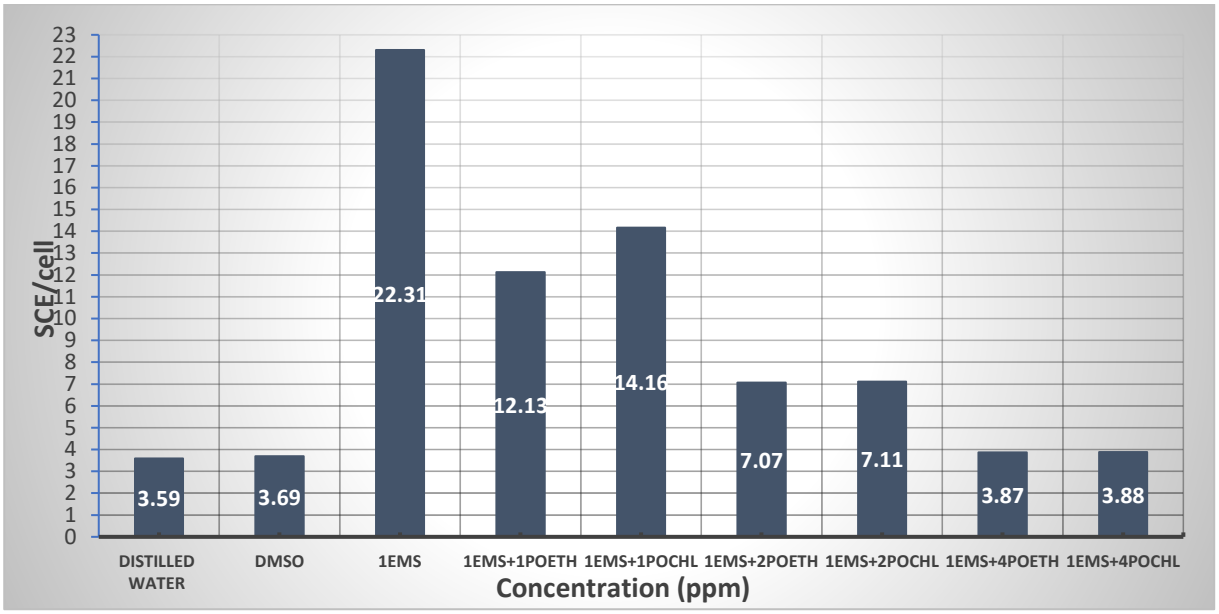

Figure c. SCE/Cell data of EMS, PO$_{eth}$ and PO$_{chl}$ application groups

SCE findings obtained as a result of PO$_{eth}$ and PO$_{chl}$ application against MMC

To observe the effects of MMC+PO$_{eth}$ and MMC+PO$_{chl}$ on SCE, 1 ppm MMC was applied to blood cultures at twice and four times the concentration (1:1/v:v, 1:2/v:2v, 1:4/v:4v). The obtained SCE frequencies and RI values are presented in Table 4.

The observed SCE/Cell ratios were 10,92±0,06, 10,75±0,03, 5,32±0,03, 5,17±0,02, 3,57±0,02 and 3,54±0,04 for 1MMC+1PO$_{eth}$, 1MMC+1PO$_{chl}$, 1MMC+2PO$_{eth}$, 1MMC+2PO$_{chl}$, 1MMC+4PO$_{eth}$ and 1MMC+4PO$_{chl}$, respectively (Table 4). The decrease in SCE/Cell ratios was significant for antigenotoxicity (P<0.05) (Figure d).

For the antigenotoxicity study, RI was also calculated in the application groups. RI values for 1MMC+1PO$_{eth}$, 1MMC+1PO$_{chl}$, 1MMC+2PO$_{eth}$, 1MMC+2PO$_{chl}$, 1MMC+4PO$_{eth}$ and 1MMC+4PO$_{chl}$ were 2,52±0,03, 2,49±0,07, 2,53±0,06, 2,54±0,01, 2,54±0,07 and 2,55±0,05, respectively (Table 4). Although RI values increased compared to the DMSO control group, the results were statistically insignificant at P>0.05.

Table 4. SCE, RI values and statistical analysis results of MMC, PO_{eth} and PO_{chl} application groups

Application groups	1st donor	2nd donor	3rd donor	SCE/Cell (Average)	Min.-Max. SCE	M1	M2	M3	RI
Distilled water	3,64	3,55	3,57	3,59±0,05	1-11	45	87	168	2,41±0,04
DMSO (%1)	3,76	3,60	3,72	3,69±0,07	1-10	46	95	146	2,25±0,07
MMC (1ppm)	28,67	27,82	25,05	27,18±0,05 [a]	11-45	45	87	128	2,01±0,01
1MMC+1PO_{eth}	10,11	11,06	11,58	10,92±0,06 [b]	1-16	45	89	178	2,52±0,03
1MMC+1PO_{chl}	10,13	10,12	12,01	10,75±0,03 [b]	1-15	45	91	173	2,49±0,07
1MMC+2PO_{eth}	5,07	5,16	5,72	5,32±0,03 [b]	1-11	44	92	176	2,53±0,06
1MMC+2PO_{chl}	5,13	5,18	5,19	5,17±0,02 [b]	1-11	45	92	177	2,54±0,01
1MMC+4PO_{eth}	3,14	3,72	3,85	3,57±0,02 [b]	1-10	45	91	178	2,54±0,07
1MMC+4PO_{chl}	3,88	3,18	3,56	3,54±0,04 [b]	1-10	46	92	179	2,55±0,05

Min.=minimum, Max.=maximum, [a]=Significant at 0.05 level compared to DMSO, [b]=Significant at 0.05 level according to MMC, P=0,05

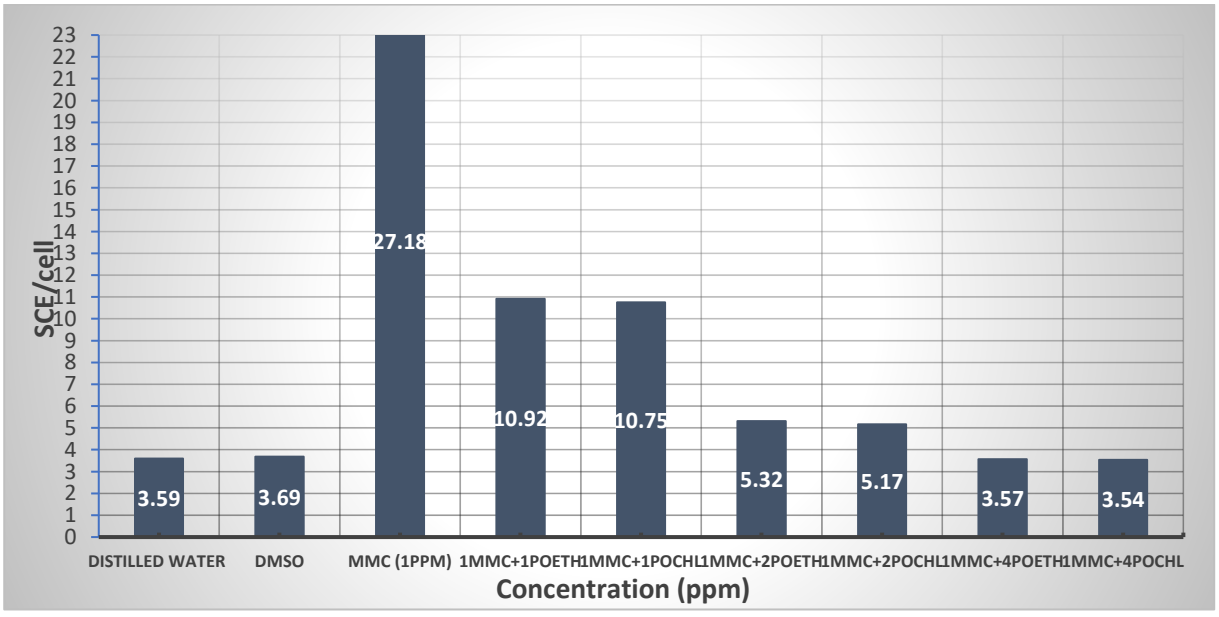

Figure d. SCE/Cell data of MMC, PO_{eth} and PO_{chl} application groups

SCE findings obtained as a result of PO_{eth} and PO_{chl} application against ENU

To observe the effects of ENU+PO_{eth} and ENU+PO_{chl} on SCE, 1 ppm ENU was applied to blood cultures at twice and four times the concentration (1:1/v:v, 1:2/v:2v, 1:4/v:4v). The obtained SCE frequencies and RI values are presented in Table 5.

The observed SCE/Cell ratios were 11,25±0,05, 11,85±0,02, 5,32±0,03, 5,56±0,05, 3,80±0,07 and 3,69±0,05 for 1ENU+1PO_{eth}, 1ENU+1PO_{chl}, 1ENU+2PO_{eth}, 1ENU+2PO_{chl}, 1ENU+4PO_{eth} and 1ENU+4PO_{chl}, respectively (Table 5). The decrease in SCE/Cell ratios was significant for antigenotoxicity ($P<0.05$) (Figure e).

For the antigenotoxicity study, RI was also calculated in the application groups. RI values for 1ENU+1PO_{eth}, 1ENU+1PO_{chl}, 1ENU+2PO_{eth}, 1ENU+2PO_{chl}, 1ENU+4PO_{eth} and 1ENU+4PO_{chl} were 2,51±0,03, 2,50±0,06, 2,49±0,01, 2,53±0,03, 2,53±0,03 and 2,54±0,05, respectively (Table 5). Although RI values increased compared to the DMSO control group, the results were statistically insignificant at $P>0.05$.

Table 5. SCE, RI values and statistical analysis results of ENU, PO_{eth} and PO_{chl} application groups

Application groups	1st donor	2nd donor	3rd donor	SCE/Cell (Average)	Min.-Max. SCE	M1	M2	M3	RI
Distilled water	3,64	3,55	3,57	3,59±0,05	1-11	45	87	168	2,41±0,04
DMSO (%1)	3,76	3,60	3,72	3,69±0,07	1-10	46	95	146	2,25±0,07
ENU (1ppm)	21,01	22,32	20,15	21,16±0,04[a]	5-40	46	90	130	2,05±0,03
1ENU+1PO_{eth}	11,11	11,26	11,37	11,25±0,05[b]	1-17	45	89	177	2,51±0,03
1ENU+1PO_{chl}	13,10	11,13	11,31	11,85±0,02[b]	1-16	44	90	175	2,50±0,06
1ENU+2PO_{eth}	5,46	5,57	5,65	5,32±0,03[b]	1-11	45	90	174	2,49±0,01

1ENU+2PO$_{chl}$	5,13	5,18	5,19	5,56±0,05 [b]	1-11	45	91	177	2,53±0,03
1ENU+4PO$_{eth}$	3,76	3,80	3,83	3,80±0,07 [b]	1-10	45	91	177	2,53±0,03
1ENU+4PO$_{chl}$	3,76	3,60	3,72	3,69±0,05 [b]	1-10	45	91	178	2,54±0,05
Min.=minimum, Max.=maximum, [a]=Significant at 0.05 level compared to DMSO, [b]=Significant at 0.05 level according to ENU, P=0,05									

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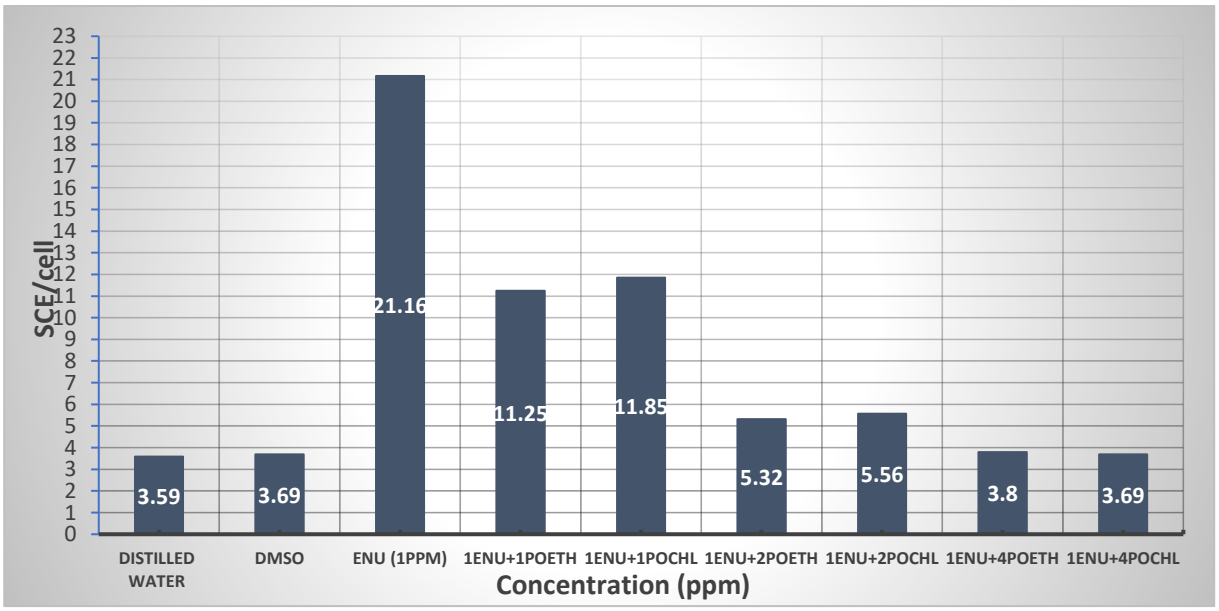

326

327 **Figure e.** SCE/Cell data of MMC, PO$_{eth}$ and PO$_{chl}$ application groups

328 **MN findings of control and purslane extracts**

329 In this study, MN was used as the second genotoxicity test. MN percentage was found as 0.73±0.09
330 in distilled water, 0.80±0.10 in DMSO, 0.77±0.06 in 1 ppm PO$_{eth}$ and 0.80±0.70 in 1 ppm PO$_{chl}$ in
331 control groups (Figure f). The difference between distilled water, 1% DMSO and purslane extracts
332 (P>0.05) was not significant. For this reason, statistical comparison was made with DMSO, which
333 was the solvent of all treatment groups.

334 NBI values for distilled water, DMSO, 1 ppm PO$_{eth}$ and 1 ppm PO$_{chl}$ were 1.51±0.06, 1.53±0.10,
335 1.55±0.03 and 1.51±0.09, respectively (Table 6) (P>0.05).

336

337 **Table 6. MN and NBI values of control groups in human peripheral lymphocyte cells**

Application Groups	Number of BNC Examined	1 MN	2 MN	3 MN	MN (%)	NDI
Distilled water	3000	22	-	-	0,73±0,09	1,51±0,06
DMSO (%1)	3000	24	-	-	0,80±0,10	1,53±0,10
PO_{eth} (1ppm)	3000	23	-	-	0,77±0,06	1,55±0,03
PO_{chl} (1ppm)	3000	24	-	-	0,80±0,70	1,51±0,09
P=0.05, BNC: binucleated cell, NDI: Nuclear Division Index						

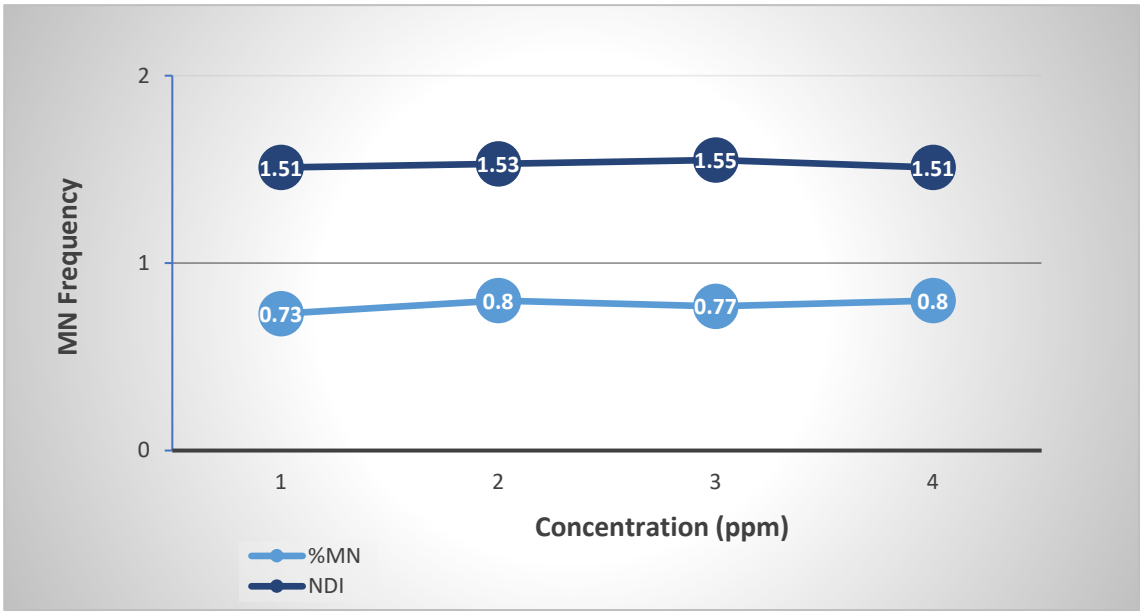

Figure f. MN% and NDI data of control groups

MN findings of EMS, MMC and ENU

In the study, MN percentages for 1 ppm EMS, 1 ppm MMC and 1 ppm ENU were 4.90±0.08, 5.37±0.06 and 4.37±0.03, respectively, and were found to be significant at P<0.05 level when compared with DMSO (Table 7). In EMS, MMC and ENU applications, 1-, 2- and 3-member MN's were observed, and the highest number was detected in MMC (122 MN).

NBI values calculated to determine the cytotoxic effect were 1.29±0.07, 1.20±0.04 and 1.30±0.06 for EMS, MMC and ENU application groups, respectively (Table 7). When compared with DMSO, it was found to be significant at P<0.05 level (Figure g).

Table 7. MN and NBI values created by EMS, MMC and ENU in human peripheral lymphocyte cells

Application Groups	Number of BNC Examined	1 MN	2 MN	3 MN	MN (%)	NDI
Distilled water	3000	22	-	-	0,73±0,09	1,51±0,06
DMSO (%1)	3000	24	-	-	0,80±0,10	1,53±0,10
EMS (1ppm)	3000	92	14	9	4,90±0,08 [a]	1,29±0,07 [a]
MMC (1ppm)	3000	94	17	11	5,37±0,06 [a]	1,20±0,04 [a]
ENU (1ppm)	3000	90	10	7	4,37±0,03 [a]	1,30±0,06 [a]
P=0.05, BNC: binucleated cell, NDI: Nuclear Division Index, [a]=Significant at the 0.05 level compared to DMSO.						

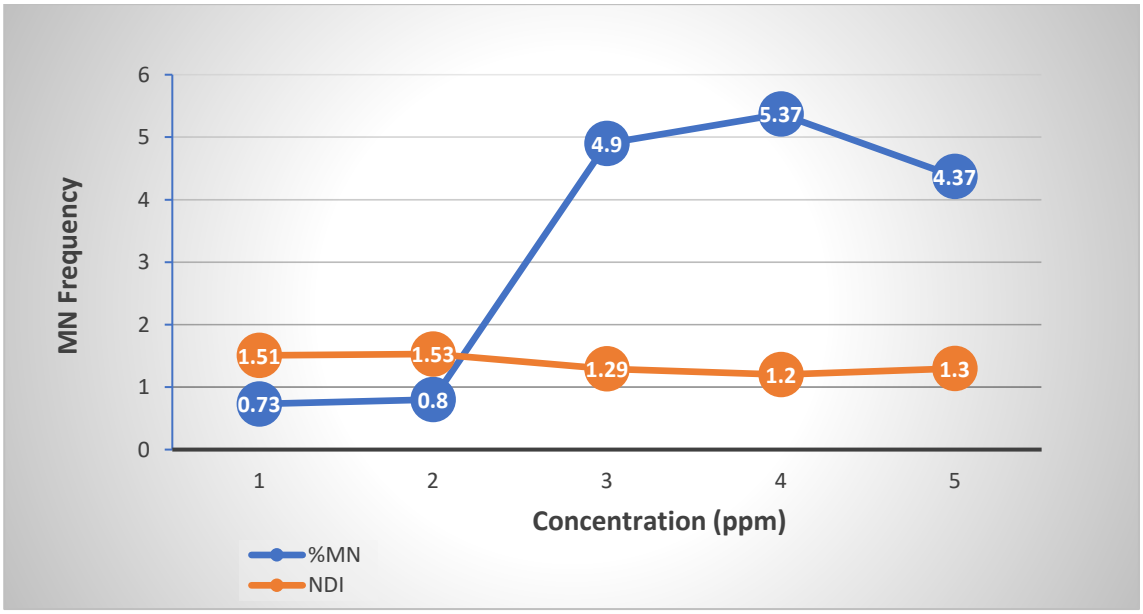

Figure g. %MN and NDI data of EMS, MMC and ENU

MN findings obtained as a result of PO_{eth} and PO_{chl} application against EMS

To observe the effects of EMS+PO_{eth} and EMS+PO_{chl} on MN, 1 ppm EMS was applied to blood cultures at two times and four times the concentrations (1:1/v:v, 1:2/v:2v, 1:4/v:4v). The obtained MN percentages and NBI values are presented in Table 8.

The observed MN percentages were 2.27±0.05, 2.10±0.07, 1.80±0.07, 1.80±0.08, 1.10±0.01 and 1.07±0.06 for 1EMS+1PO_{eth}, 1EMS+1PO_{chl}, 1EMS+2PO_{eth}, 1EMS+2PO_{chl}, 1EMS+4PO_{eth} and 1EMS+4PO_{chl}, respectively (Figure h). The decrease in MN percentages was significant for antigenotoxicity ($P<0.05$).

For the antigenotoxicity study, NBI was also calculated in the application groups. NBI values for 1EMS+1PO_{eth}, 1EMS+1PO_{chl}, 1EMS+2PO_{eth}, 1EMS+2PO_{chl}, 1EMS+4PO_{eth} and 1EMS+4PO_{chl} were 1.44±0.05, 1.43±0.03, 1.48±0.04, 1.49±0.07, 1.50±0.07 and 1.50±0.08, respectively (Table 8). NBI values approached the control group, DMSO. This improvement in cytotoxic effect was statistically significant ($P<0.05$).

Table 8. MN and NBI values created by EMS, PO_{eth} and PO_{chl} in human peripheral lymphocyte cells

Application Groups	Number of BNC Examined	1 MN	2 MN	3 MN	MN (%)	NDI
Distilled water	3000	22	-	-	0,73±0,09	1,51±0,06
DMSO (%1)	3000	24	-	-	0,80±0,10	1,53±0,10
EMS (1ppm)	3000	92	14	9	4,90±0,08 [a]	1,29±0,07 [a]
1EMS+1PO_{eth}	3000	66	1	-	2,27±0,05[b]	1,44±0,05[b]
1EMS+1PO_{chl}	3000	63	-	-	2,10±0,07[b]	1,43±0,03[b]
1EMS+2PO_{eth}	3000	54	-	-	1,80±0,07[b]	1,48±0,04[b]

1EMS+2PO$_{chl}$	3000	54	-	-	1,80±0,08[b]	1,49±0,07[b]
1EMS+4PO$_{eth}$	3000	33	-	-	1,10±0,01[b]	1,50±0,07[b]
1EMS+4PO$_{chl}$	3000	32	-	-	1,07±0,06[b]	1,50±0,08[b]
P=0.05, BNC: binucleated cell, NDI: Nuclear Division Index, [a]=Significant at the 0.05 level compared to DMSO, [b]=Significant at the 0.05 level compared to EMS.						

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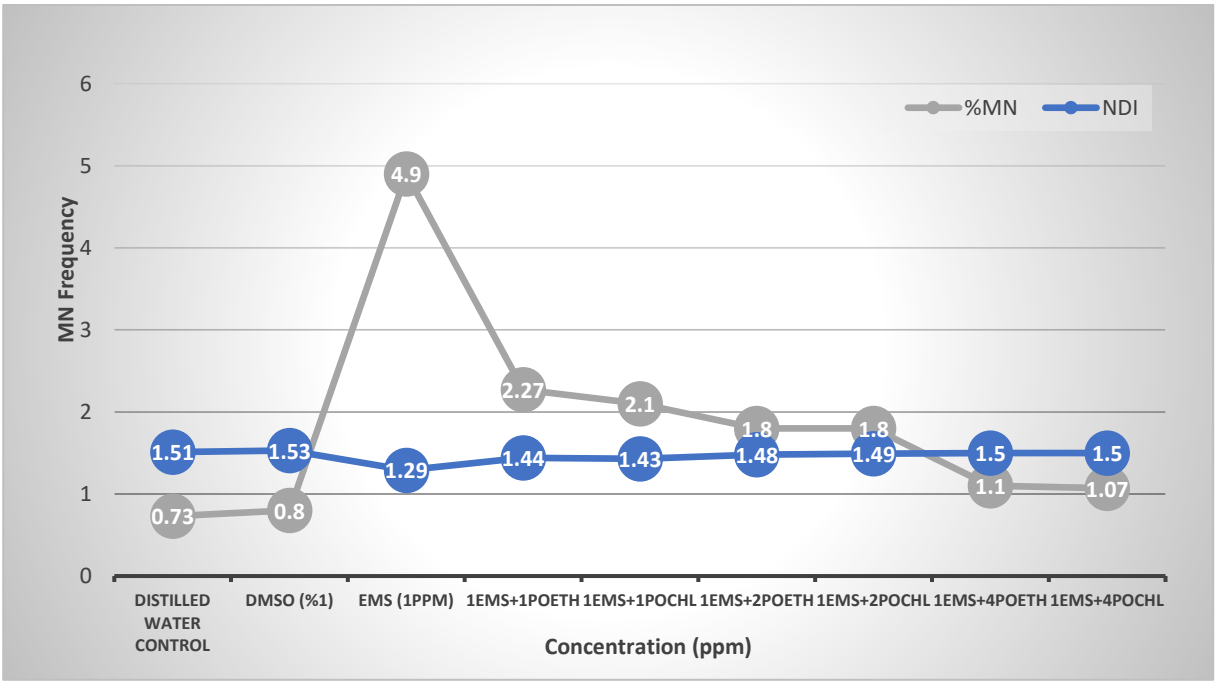

374

375 **Figure h.** %MN and NDI data of PO$_{eth}$ and PO$_{chl}$ against EMS

376 **MN findings obtained as a result of PO$_{eth}$ and PO$_{chl}$ application against MMC**

377 To observe the effects of MMC+PO$_{eth}$ and MMC+PO$_{chl}$ on MN, 1 ppm MMC was applied to blood
378 cultures at two times and four times the concentrations (1:1/v:v, 1:2/v:2v, 1:4/v:4v). The obtained MN
379 percentages and NBI values are presented in Table 9.

380 The observed MN percentages were 2,47±0,01, 2,37±0,07, 1,83±0,05, 1,80±0,05, 1,20±0,02 and
381 1,10±0,05 for 1MMC+1PO$_{eth}$, 1MMC+1PO$_{chl}$, 1MMC+2PO$_{eth}$, 1MMC+2PO$_{chl}$, 1MMC+4PO$_{eth}$ and
382 1MMC+4PO$_{chl}$, respectively (Figure i). The decrease in MN percentages was significant for
383 antigenotoxicity (P<0.05).

384 For the antigenotoxicity study, NBI was also calculated in the application groups. NBI values for
385 1MMC+1PO$_{eth}$, 1MMC+1PO$_{chl}$, 1MMC+2PO$_{eth}$, 1MMC+2PO$_{chl}$, 1MMC+4PO$_{eth}$ and

1MMC+4PO_{chl} were 1,40±0,02, 1,41±0,03, 1,47±0,05, 1,49±0,06, 1,49±0,05 and 1,50±0,02, respectively (Table 9). NBI values approached the control group, DMSO. This improvement in cytotoxic effect was statistically significant (P<0.05).

Table 9. MN and NBI values created by MMC, PO_{eth} and PO_{chl} in human peripheral lymphocyte cells

Application Groups	Number of BNC Examined	1 MN	2 MN	3 MN	MN (%)	NDI
Distilled water	3000	22	-	-	0,73±0,09	1,51±0,06
DMSO (%1)	3000	24	-	-	0,80±0,10	1,53±0,10
MMC (1ppm)	3000	94	17	11	5,37±0,06[a]	1,20±0,04[a]
1MMC+1PO_{eth}	3000	70	2	-	2,47±0,01[b]	1,40±0,02[b]
1MMC+1PO_{chl}	3000	69	1	-	2,37±0,07[b]	1,41±0,03[b]
1MMC+2PO_{eth}	3000	55	-	-	1,83±0,05[b]	1,47±0,05[b]
1MMC+2PO_{chl}	3000	54	-	-	1,80±0,05[b]	1,49±0,06[b]
1MMC+4PO_{eth}	3000	36	-	-	1,20±0,02[b]	1,49±0,05[b]
1MMC+4PO_{chl}	3000	33	-	-	1,10±0,05[b]	1,50±0,02[b]

P=0.05, BNC: binucleated cell, NDI: Nuclear Division Index, [a]=Significant at the 0.05 level compared to DMSO, [b]=Significant at the 0.05 level compared to MMC.

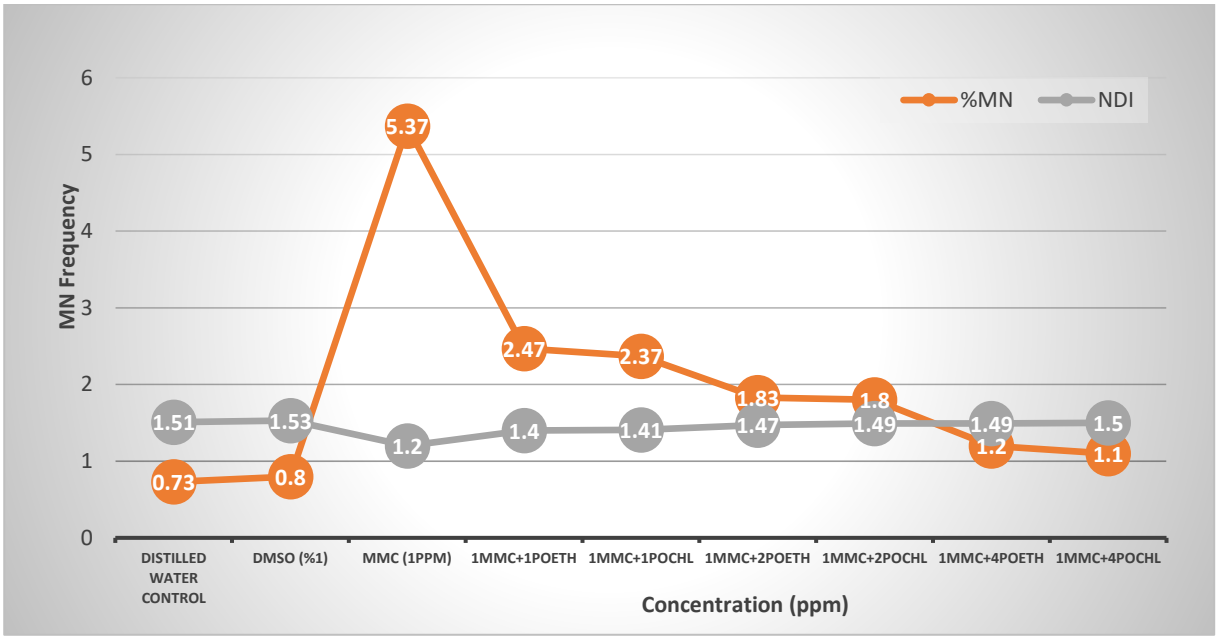

Figure i. %MN and NDI data of PO_{eth} and PO_{chl} against MMC

MN findings obtained as a result of PO_{eth} and PO_{chl} application against ENU

To observe the effects of ENU+PO_{eth} and ENU+PO_{chl} on MN, 1 ppm ENU was applied to blood cultures at two times and four times the concentrations (1:1/v:v, 1:2/v:2v, 1:4/v:4v). The obtained MN percentages and NBI values are presented in Table 10.

The observed MN percentages were 2,17±0,06, 2,17±0,05, 1,73±0,04, 1,70±0,07, 1,10±0,06 and 1,03±0,03 for 1ENU+1PO_{eth}, 1ENU+1PO_{chl}, 1ENU+2PO_{eth}, 1ENU+2PO_{chl}, 1ENU+4PO_{eth} ve 1ENU+4PO_{chl}, respectively (Figure j). The decrease in MN percentages was significant for antigenotoxicity ($P<0.05$).

For the antigenotoxicity study, NBI was also calculated in the application groups. NBI values for 1ENU+1PO_{eth}, 1ENU+1PO_{chl}, 1ENU+2PO_{eth}, 1ENU+2PO_{chl}, 1ENU+4PO_{eth} ve 1ENU+4PO_{chl} were 1,43±0,07, 1,43±0,08, 1,45±0,04, 1,45±0,05, 1,49±0,04 and 1,50±0,03, respectively (Table 10). NBI values approached the control group, DMSO. This improvement in cytotoxic effect was statistically significant ($P<0.05$).

Table 9. MN and NBI values created by ENU, PO_{eth} and PO_{chl} in human peripheral lymphocyte cells

Application Groups	Number of BNC Examined	1 MN	2 MN	3 MN	MN (%)	NDI
Distilled water	3000	22	-	-	0,73±0,09	1,51±0,06
DMSO (%1)	3000	24	-	-	0,80±0,10	1,53±0,10
ENU (1ppm)	3000	90	10	7	4,37±0,03 [a]	1,30±0,06 [a]
1ENU+1PO$_{eth}$	3000	65	-	-	2,17±0,06 [b]	1,43±0,07 [b]
1ENU+1PO$_{chl}$	3000	65	-	-	2,17±0,05 [b]	1,43±0,08 [b]
1ENU+2PO$_{eth}$	3000	52	-	-	1,73±0,04 [b]	1,45±0,04 [b]
1ENU+2PO$_{chl}$	3000	51	-	-	1,70±0,07 [b]	1,45±0,05 [b]
1ENU+4PO$_{eth}$	3000	33	-	-	1,10±0,06 [b]	1,49±0,04 [b]
1ENU+4PO$_{chl}$	3000	31	-	-	1,03±0,03 [b]	1,50±0,03 [b]
P=0.05, BNC: binucleated cell, NDI: Nuclear Division Index, [a]=Significant at the 0.05 level compared to DMSO, [b]=Significant at the 0.05 level compared to ENU.						

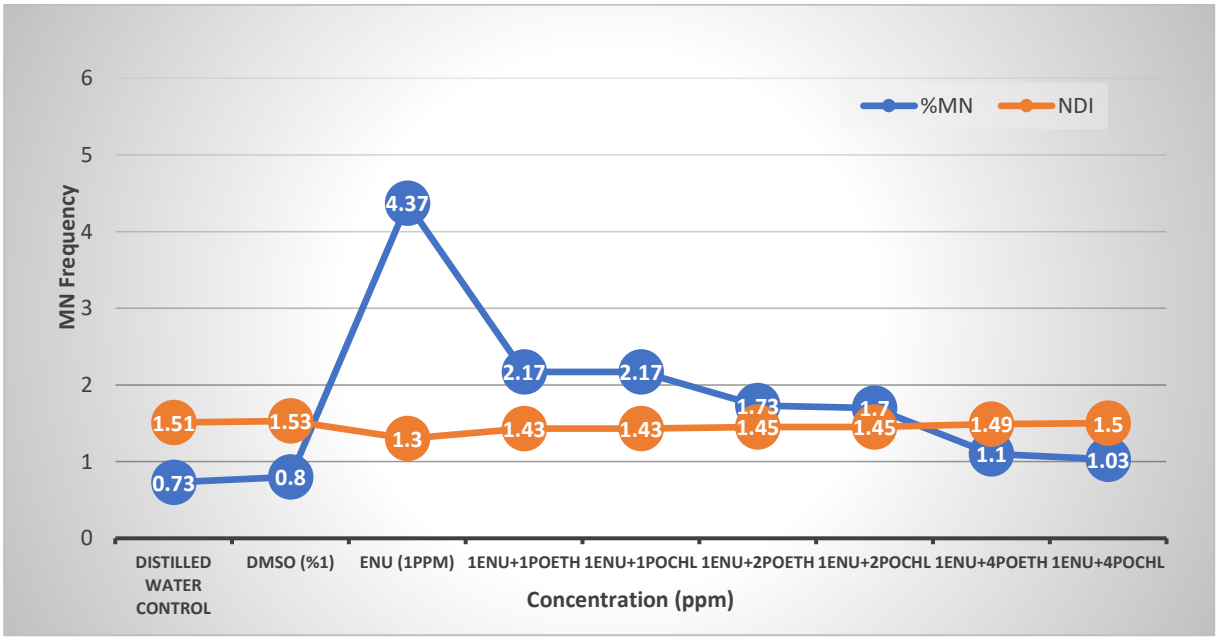

Figure j. %MN and NDI data of PO$_{eth}$ and PO$_{chl}$ against ENU

Discussion

Genotoxic chemicals are substances that can cause permanent changes in genetic material by directly or indirectly damaging DNA. Among these chemicals, Ethyl Methane Sulfonate (EMS), Mitomycin-C (MMC) and N-Nitroso-N-ethylurea (ENU) are widely used in research and are well-known agents. Each of them causes genotoxicity with different mutation mechanisms. In this study, the antigenotoxic effects of ethanol (PO_{eta}) and chloroform (PO_{chl}) extracts of *Portulaca oleracea* (purslane) against genotoxic agents such as EMS, MMC and ENU were evaluated in human peripheral lymphocyte cells using the Sister Chromatid Exchange (SCE) Test and the Micronucleus (MN) Test. While there was no statistically significant difference ($P>0.05$) between the negative control groups (distilled water, DMSO, PO_{eta} and PO_{chl}), the difference in genotoxic (average SCE and %MN) and cytotoxic (Replication Index and Nuclear Division Index) values of the positive controls (EMS, MMC, ENU) was statistically significant ($P<0.05$).

The genotoxic effects of EMS, MMC and ENU occur through different mechanisms. While EMS and ENU cause mutations by adding alkyl groups to DNA bases, MMC mostly causes genomic instability by forming cross-links in DNA. Studies on the genotoxic effects of these chemicals are important, especially in terms of understanding carcinogenicity, mutagenicity and genetic diseases. While alkylating agents such as EMS and ENU cause mutations by covalently binding to DNA bases, MMC forms DNA cross-links. EMS and ENU usually cause point mutations. ENU and EMS are ethylating agents, but their chemical properties are different. EMS has a high Swain-Scott substrate constant (S) and reacts predominantly with ring nitrogen atoms in DNA (high nucleophilicity), while ENU, which has a lower S value, reacts significantly with oxygen atoms. Alkylated oxygen atoms, especially O^6-alkylguanine, play an important role in the induction of gene mutations. O^6-alkylguanine can pair with thymine to lead to GC to AT transitions (52–54). EMS causes incorrect base pairing by adding ethyl groups to guanine and thymine bases in DNA. O^6-ethylguanine bases formed as a result of ethylation are incorrectly paired during replication and cause G:C→A:T transition mutations. The mutation mechanism of EMS is particularly prone to creating transition mutations (55). Recent studies have shown that EMS has potential adverse effects such as cancer, birth anomalies and

hereditary effects (56–58). ENU, on the other hand, ethylates DNA bases by adding ethyl groups, especially at the O^2 and O^4 positions of adenine. This leads to A:T → T:A and G:C → A:T transition mutations (59–61). ENU, high mutation frequencies and genetic instability are observed in its applications. It also causes DNA strand breaks and oxidative damage (19). The toxicity of these agents is also associated with the excessive production of reactive oxygen species (ROS). It shows its mutagenic activity by affecting oxygen or nitrogen atoms in DNA bases (62). MMC, on the other hand, causes replication fork collapse and double-strand breaks by forming interstrand cross-links that physically block replication and transcription (12). These cross-links can inhibit DNA replication and transcription and disrupt genomic stability. MMC can particularly trigger double-strand breaks and chromosomal aberrations. The genotoxic effects of MMC are cell cycle dependent and are mostly more effective in G1 and S phases (63). In addition, it causes oxidative damage by producing reactive oxygen species (ROS) through the redox cycle (13,14). It is also known to cause oxidative damage in the cell by cross-linking with DNA and adding an alkyl group (64). It also produces interstrand cross-links (ICL's) that stop replication forks. Insoluble ICL's cause chromatid exchanges and ROS-mediated oxidative damage (12,14,15). MMC is often used as a powerful anticancer agent in cancer treatment and has mutagenic and carcinogenic properties that cause secondary cancer (16). Studies in endothelial cells show that genotoxic stress caused by MMC causes cell cycle arrest and cell death (65).

The effects of genotoxic agents (EMS, MMC and ENU) used in the study on DNA are consistent with the literature. Statistically significant increases were observed in the frequencies of SCE and MN in the groups where EMS, MMC and ENU were applied alone ($P<0.05$). For example, the average SCE values in the group applied EMS, MMC and ENU were measured as 22.31±0.03, 27.18±0.05 and 21.16±0.04, respectively, which revealed a significant genotoxic effect compared to the control group (DMSO: 3.69±0.07). Similarly, MN percentages were also significantly increased by genotoxic agents (DMSO: 0.80±0.10%, EMS: 4.90±0.08%; MMC: 5.37±0.06%; ENU: 4.37±0.03%). These findings confirm the damage mechanisms of these agents on DNA and chromosomes.

In the groups where PO_{eth} and PO_{chl} extracts were applied alone, no statistically significant difference was found in the values of SCE and MN compared to the control groups (distilled water and DMSO) ($P>0.05$). These results show that purslane extracts do not have direct genotoxic and cytotoxic effects. This finding is consistent with the studies in the literature that purslane is rich in antioxidant components (flavonoids, omega-3 fatty acids, ascorbic acid) and does not cause DNA damage (66,67).

Therefore, when purslane extracts were applied together with genotoxic agents, a significant decrease in the frequencies of SCE and MN was observed in a dose-dependent manner. For example, PO_{eth} applied together with EMS decreased the SCE value from 22.31±0.03 to 3.87±0.05. Similarly, the percentages of MN were also significantly reduced (EMS+4PO_{eth}: 1.10±0.01%; EMS+4PO_{chl}: 1.07±0.06%). This effect can be explained by the antioxidant and DNA repair mechanism activating properties of purslane extracts. Flavonoids and phenolic compounds contained in purslane can reduce oxidative stress by neutralizing free radicals (7,68,69).

The antigenotoxic effects of Portulaca oleracea against EMS, MMC and ENU may be associated with its high antioxidant capacity. Purslane is rich in components such as flavonoids, betalains and ascorbic acid, which can reduce oxidative stress by neutralizing free radicals (70). The reduction of mutations caused by these genotoxic agents by purslane extracts can probably be explained by their ability to scavenge reactive oxygen species (ROS) or activate DNA repair mechanisms. Components such as ascorbic acid and α-tocopherol can prevent DNA damage by neutralizing ROS (71). In particular, betalains have been shown to prevent the formation of DNA adducts induced by alkylating agents in experimental models. Similarly, Oliveira et al. (72) attributed the hepatoprotective effect of *Portulaca oleracea* to its antioxidant activity. In this study, the decrease in the effect with increasing doses of extracts may be associated with the emergence of pro-oxidant effects at high concentrations. This is consistent with the literature on the paradoxical behavior of antioxidants (73). The decrease in mean SCE, SCE/cell and %MN values in PO_{eth} and PO_{chl} groups indicates that the antigenotoxic effect becomes more pronounced as the extract concentration increases. The differences in the efficacy of

494 PO_{eth} and PO_{chl} extracts may be related to the polarity of the extraction solvents. While ethanol better
495 captures polar compounds (flavonoids, phenolic acids), chloroform captures lipophilic compounds
496 (terpenoids, fatty acids) (74). In this study, the strong antigenotoxic effect of purslane against EMS
497 and ENU can be explained by the superiority of flavonoids in reducing alkylation damage. In contrast,
498 its effectiveness against MMC can be associated with the ability of terpenoids to inhibit DNA cross-
499 linking (12,75–77). It has been reported that *Portulaca oleracea* extracts increase the expression of
500 enzymes such as glutathione-S-transferase (GST) and superoxide dismutase (SOD) by stimulating
501 the Nrf2/ARE pathway (78,79). These enzymes play a critical role in the detoxification of
502 electrophilic carcinogens such as EMS and ENU. Therefore, the reduction of genotoxicity by the
503 extracts can be explained not only by the antioxidant effect but also by the modulation of cellular
504 defense mechanisms. These findings emphasize that the chemical diversity of plant extracts is
505 decisive in pharmacological effects. For example, Ethanol and acetone extracts of *Portulaca oleracea*
506 showed strong DPPH, ABTS and hydroxyl radical scavenging activities while chloroform and n-
507 hexane extracts showed significant superoxide radical scavenging activity (80).
508 In the study, the antigenotoxic effect became more pronounced as the extract concentration increased,
509 supporting the dose-response relationship. However, the plateauing or slight decrease in the effect at
510 high doses may indicate the pro-oxidant behavior of antioxidants. This paradox is explained by the
511 interaction of antioxidants with metal ions and the production of ROS, especially at high
512 concentrations (81). Similarly, there are studies in which *Portulaca oleracea* extracts reacted with
513 Fe^{2+} ions and produced hydroxyl radicals (82). This situation reveals the importance of determining
514 the optimal dose range of extracts.
515
516 **Conclusions**
517
518 This study confirms the antigenotoxic and anticytotoxic effects of *Portulaca oleracea* extracts against
519 genotoxic and cytotoxic agents. Especially the dose-response relationship and the diversity of

components support the use of this plant as a protective agent. It was revealed that ethanol and chloroform extracts of *Portulaca oleracea* significantly reduced the toxicity caused by EMS, MMC and ENU. The effect became more pronounced especially with the increase in dose, emphasizing the protective potential of these extracts. The obtained findings are promising that dietary intake of purslane or its pharmacological formulations can be used to prevent DNA damage in case of exposure to genotoxic chemicals.

Declarations

Ethics approval and consent to participate: This study complies with all provisions of the Declaration of Helsinki. Informed consent was obtained from all participants in the study. It was approved by the Aydın Adnan Menderes University Faculty of Medicine, Non-Interventional Clinical Research Ethics Committee (DECISION:02 Protocol No: 2025/120).

The area where the *Portulaca oleracea* samples were collected is public land belonging to the Non-Timber Forest Products and Services Department of the General Directorate of Forestry, Ministry of Forestry and Water Affairs of the Republic of Turkey, and is not subject to any permit (CIRCULAR NO: 302 INVENTORY AND PLANNING OF NON-TIMBER FOREST PRODUCTS AND PRINCIPLES OF PRODUCTION AND SALE). A sample of this material is not kept in a publicly accessible herbarium.

Consent for publication: All of the material is owned by the authors and/or no permissions are required.

Availability of data and materials: The data that support the findings of this study are available from the corresponding author, [HK], upon reasonable request.

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