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Essential oil from *Pelargonium graveolens* shows anticancer activity and ameliorates the DNA damage and genotoxicity of doxorubicin in mice

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Abstract

Pelargonium graveolens essential oil (PGEO) is an interesting plant extract. The ability of this oil to protect against genotoxicity induced by the anticancer drug doxorubicin (DOX) and its anticancer activity were examined. The following groups of mice were tested for genotoxicity: Group I, negative control; Group II, orally treated with 0.9 ml/kg PGEO; Group III, i.p. injected with DOX at 20 mg/kg; Groups IV–VI, animals received PGEO doses of 0.3, 0.6, and 0.9 ml/kg orally for 5 days, plus a single i.p. injection of DOX on the 5th day of treatment. Significant genotoxicity after DOX treatment was noted in bone marrow cells evidenced by increasing frequency of micronuclei, sister chromatid exchanges, and chromosomal abnormalities. Conversely, DOX did not affect spermatocyte chromosomes. PGEO showed no effects on any genotoxicity endpoints examined. Further, the oil was protective against genotoxicity induced by DOX. PGEO also displayed strong cytotoxic effects on all human cancer cell lines tested: HepG2, PC3, A549, A431, HCT116, and MCF7, with ~100% cell death at a concentration of 100 µg/ml. GC/MS analysis identified 27 compounds. The two major components were citronellol and geraniol which participate to a great extent with other components in the protective role of PGEO.

Keywords: Doxorubicin, Genotoxicity, *Pelargonium graveolens*, Essential oil, Protective role, Anticancer effect..

1. Introduction

Herbal medicines and their secondary metabolites are attractive natural products for many pharmacologists who working to produce new drugs [1, 2]. Essential oils are folk medicines, and their pharmaceutical uses have expanded worldwide. These oils are recognized as safe and display a variety of biological and medicinal properties [3]. Monoterpenes are the major components of the essential oils from many plants and are known for their natural antioxidant, anti-inflammatory, and even anticancer potential [4]. The most interesting essential oil is produced by *Pelargonium graveolens*, commonly known as rose-scented geranium. Traditionally, the plant has been used to treat a variety of symptoms, including nephritis, wounds, fever, hemorrhoids, gastrointestinal diseases, hyperglycemia, tuberculosis, inflammation, and cancer [5]. The essential oil of *P. graveolens* (PGEO) shows antibacterial, antimicrobial, and antifungal activities and is used in the food industry as a preservative agent [6]. It is currently used by the French medical community for the treatment of diabetes, diarrhea, gastric ulcers, gallbladder issues, urinary stones, liver dysfunction, and sterility [7].

Chemotherapeutic drugs adversely affect cellular components and interfere with DNA and RNA synthesis. These undesirable side effects are caused by the over-production of reactive oxygen species (ROS) and the consequent imbalance of oxidation–reduction (redox) potential. Doxorubicin (Adriamycin, DOX) is an anthracycline medication and an antitumor antibiotic available for the treatment of a broad spectrum of human cancers. The drug is administered either in combination with other antitumor drugs or in combination with surgery and radiation [8]. However, patients receiving DOX therapy show genotoxicity and myelosuppression. These side effects may lead to secondary malignancy and dose-dependent cardiotoxicity [9]. DOX induces genotoxicity by inhibiting DNA topoisomerase and generating free radicals [10]. Secondary malignances, cardiomyopathy and cardiac failure may develop many years after the cessation of cancer treatment and tumor remission [11].

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The present work evaluated the protective role of PGEO against DNA damage and genotoxicity caused by DOX. Further, the anticancer activity of the oil was tested in several human cancer cell lines. Gas chromatography/mass spectrometry (GC/MS) analysis was used to identify active components of the oil. Finally, the study discusses the biological activities of primary oil components to explore novel crop additive value.

Materials and methods

Plant material

Pelargonium graveolens was collected from Cairo university farming, Giza Governorate, during 2019. Specimens were identified by Dr. Adel Salama (voucher specimens 345) Medicinal and Aromatic Plants Research Department, National Research Centre, Dokki, Giza, Egypt.

Chemical investigation of essential oil

Determination

The volatile oil content of aerial parts was assessed by extracting oil using hydro-distillation for 3 h in a Clevenger's apparatus [12]. The oily layer obtained on top of the aqueous distillate was separated and dried with anhydrous sodium sulfate (0.5 g). This essential oil was kept in sealed air-tight glass vials and covered with aluminum foil at 4°C until further analysis. Extraction was performed in triplicate, and oil content was recorded as the mean of the extractions as a percent (w/w).

Chemical composition of PGEO

GC/MS analysis

The GC/MS system (Agilent Technologies) was equipped with a gas chromatograph (7890B) and a mass spectrometer detector (5977A) at Central Laboratories Network, National Research Centre, Cairo, Egypt. Samples were diluted with hexane (1:19, v/v). The GC was equipped with an HP-5MS column (30 m × 0.25 mm internal diameter and 0.25 µm film thickness). Analyses used helium as the carrier gas at a flow rate of 1.0 ml/min with a split ratio of 1:30, injection volume of 1 µl, and the following temperature regime: 40°C for 1 min; rising at 4°C/min to 150°C and held for 6 min; and rising at 4°C/min to 210 °C and held for 1 min. The injector and detector were held at 280°C and 220°C, respectively. Mass spectra were obtained by electron ionization at 70 eV, using a spectral range of m/z 50–550. Identification of constituents used comparisons of spectrum fragmentation pattern with patterns stored in the Wiley and NIST Mass Spectral Library data.

Chemicals

DOX was purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals used in extraction were purchased from ADWIC (Cairo, Egypt).

In vivo studies

Animals

Male white Swiss mice (*Mus musculus*), aged 9 to 12 weeks, were used in all experiments. Animals were obtained from a closed random-bred colony at the National Research Centre (Giza, Egypt). Mice used for each experiment were of similar age (±1 week) and weight (± 2 g). Animals were housed in polycarbonate boxes with steel wire tops and bedded with wood shavings. Ambient temperature was controlled at 22°C ± 3°C with a relative humidity of 50% ± 15% and a 12 h light/dark photoperiod. Food and water were provided *ad libitum*. The experiments were conducted according to the Animal Research Ethical Committee Guidelines of the National Research Centre. The Approval Certificate is under the number: 19 163.

Experimental design

Ninety-five mice were used as follows: 35 for analysis of chromosomal aberrations in bone marrow and spermatocytes, 30 for micronuclei, and 30 for sister chromatid exchange (SCE) analysis. Mice were subdivided into groups of five for these analyses. As the following groups of mice were assigned: Group I, negative control; Group II, PGEO control orally administered at 0.9 ml/kg; Group III, positive control, i.p. injected with 20 mg/kg DOX (20 and 40 mg/kg in experiments of meiotic chromosomes), and Groups IV–VI, DOX plus PGEO (0.3, 0.6, and 0.9 ml/kg).

Cytogenetic analysis

Micronucleus test

Micronucleus preparations from bone marrow of control and treated mice followed the standard test protocol of Schmid [13] and OECD 474 Guideline for Testing Chemicals [14]. Briefly, bone marrow cells were collected from bilateral femurs by separation in 3 ml of fetal bovine serum, centrifugation, and smearing onto slides. Air-dried slides were fixed by submerging in absolute methanol for 10–20 min. Fixed slides were stained using the May Grünwald–Giemsa protocol. Micronuclei were identified as dark blue staining bodies in the cytoplasm of polychromatic erythrocytes (MNPEs). The ratio of erythrocytes to nucleated cells was determined and expressed as the percentage of PEs/100 nucleated cells (PEs + NEs). Two thousand nucleated cells were scored/animal (5 animals/group). Scoring was performed under 1000× magnification with a light microscope.

Sister chromatid exchange analysis (SCEs)

5-Bromodeoxyuridine (BrdU, Sigma) tablets weighing 55 ± 5 mg were subcutaneously implanted for 21–24 h in each mouse for SCE analysis. Colchicine was injected i.p. 2 h before bone marrow cell harvesting. Hypotonic treatment, fixation of the cells, and chromosome preparations were carried out following Fahmy et al. [15]. Five experiments with five mice were examined for each treatment, and 25

metaphases per experiment were analyzed for SCEs per cell (125 cells per treatment).

Chromosomal aberration assay in mouse bone marrow and spermatocytes

Bone marrow chromosomes were prepared as described by Fahmy et al. [16]. In brief, mouse bone marrow cells were collected from both femurs. Cells were incubated in hypotonic solution (KCL 0.075 M) for 20 min at 37°C and then centrifuged. Cell pellets were suspended in a fixative (methanol/glacial acetic acid, 3:1). This step was repeated at least twice, and then cells were suspended in a few drops of fixative and spread onto frozen slides, air-dried, stained with 10% Giemsa for 30 min, washed, and air-dried again.

Spermatocyte chromosomes were prepared from the testes of the same animals as described by Evans et al. [17] with some modifications [18]. Briefly, testes were removed and pressed into a petri dish containing an isotonic solution of 2.2% trisodium citrate. The cell suspension was then centrifuged for 5 min at 1500 rpm. The cell pellet was incubated in a hypotonic solution of 1.1% trisodium citrate for 20 min at 37°C followed by centrifugation. The cell pellet was washed twice with freshly prepared fixative. A few drops of the resulting cell suspension were placed on a clean microscopic slide, air-dried, and stained with 10% Giemsa stain. One hundred well-spread metaphases were analyzed per mouse for bone marrow and mouse spermatocytes and used to record different kinds of chromosome abnormalities (CAs). Scoring for CAs was performed under 2000× magnification with a light microscope.

Statistical analysis

Cytogenetic data were statistically analyzed using one-way analysis of variance. Evaluation of inhibitory index for the extent of protection used the formula of Madrigal-Bujaidar et al. [19]:

Inhibitory index (II) = [1 − (plant extract plus positive control − negative control) / (positive control − negative control)] × 100.

In vitro studies

Cell viability

Cell viability was assessed by the mitochondrial-dependent reduction of yellow MTT (3-(5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to purple formazan [20]. All procedures were performed in a sterile area using a laminar flow cabinet, biosafety class II level (Baker, SG403INT, Sanford, ME, USA). Cancer cells were suspended in DMEM-F12 for HePG2, MCF7, PACA2, and HCT116 and for BJ1 normal cells. The medium contained 1% antibiotic-antimycotic mixture (10,000 U/ml potassium penicillin, 10,000 µg/ml streptomycin sulfate, and 25 µg/ml amphotericin B) and 1% L-glutamine at 37°C under 5% CO_2.

Cells were batch cultured for 10 days and then seeded at a concentration of 1 × 104 cells/well in fresh complete growth medium in 96-well microtiter plastic plates at 37°C for 24 h under 5% CO_2 using a water-jacketed carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA). The medium was aspirated, a fresh medium without serum was added, and cells were incubated either alone (negative control) or with different concentrations of PGEO to final concentrations of 100, 50, 25, 12.5, 6.25, 3.125, 1.56, and 0.78 µg /ml). After 48 h, the medium was aspirated, 40 µl MTT salt (2.5 µg/ml) was added to each well, and plates were incubated for an additional 4 h at 37°C under 5% CO_2. Ten percent sodium dodecyl sulfate in deionized water (200 µl) was added to each well to stop the reaction and dissolve crystals. Plates were then incubated overnight. A positive control with 100 µg/ml PGEO, known to cause 100% lethality, was used to ensure that the total range of viability was assessed [21, 22].

Absorbance was measured using a microplate multiwell reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) at 595 nm and a reference wavelength of 620 nm. Statistical significance was assessed between treated and negative control cells using independent t-tests in the SPSS 11 program. DMSO was used for dissolution of plant extracts. Its final concentration was less than 0.2%. The percentage change in viability was calculated as follows:

(1 − (reading of extract/reading of negative control) × 100).

A probit analysis was used to estimate IC_{50} and IC_{90} again using SPSS 11.

The degree of selectivity of synthetic compounds is expressed as follows: SI = IC_{50} of pure compounds in a normal cell line/IC_{50} of the same pure compound in a cancer cell line. IC_{50} is the concentration required to kill 50% of the cell population.

but without the use of the primary antibody.

Results

Phytochemical studies

The chemical composition of PGEO was indicated by GC/MS analysis (Table 1). Twenty-seven compounds constituted 99.23% of geranium essential oil. Major components were citronellol (CT; 38.27%), geraniol (12.39%), 6-octen-1-ol, 3,7-dimethyl-formate (10.23%), l-menthone (7.26%), ɣ-eudesmol (7.14%), linalool (4.97%), and geranyl formate (2.94%). All other components were present in amounts less than 2.5%.

Genotoxic study

There was a significant increase in the frequency of MNPEs after treatment with DOX, where the percentage reached 9.71 ± 0.41 compared with 1.89 ± 0.55 for the negative control cells (Figs. 1 and 2a). Severe toxicity of DOX on bone marrow cells was

evidenced by the increasing percentage of PEs/total counted cells. This percentage reached 19.88 ± 0.39 compared with 5.27 ± 0.47 for the negative control. Animals treated with PGEO showed no adverse impacts on erythrocyte percentage or MNPEs frequency. Moreover, PGEO ameliorated the effects of DOX on micronuclei and the toxicity induced in bone marrow. PGEO actions were dose-dependent.

The frequency of SCEs is expressed as number per cell. A single i.p. treatment with DOX (20 mg/kg) induced significant frequencies of SCEs (Table 2, Fig. 2b), 34.22 ± 0.83/cell ($p < 0.01$) compared with 4.11 ± 0.59 for the negative control cells. PGEO did not induce SCEs at the concentration of 0.9 ml/kg –the highest tested level with a frequency of 4.38 ± 0.48/cell compared well with 4.11 ± 0.59 for negative control cells. Moreover, the frequency of SCEs was 19.28 ± 0.53, 17.16 ± 0.37, and 13.22 ± 0.59 after the treatment of animals with 0.3, 0.6, and 0.9 ml/kg of PGEO plus DOX (20 mg/kg), respectively, indicating a pronounced protection from DOX toxicity.

The frequency of chromosomal aberrations induced in mouse bone marrow, after treatment with DOX and PGEO indicated that the highest tested dose of PGEO (0.9 ml/kg) did not induce CAs compared with the negative control cells (Table 3). DOX (20 mg/kg) alone induced a highly significant percentage of CAs in bone marrow cells, 40.20 ± 1.73 *vs.* 2.20 ± 0.37 for control. Pretreatment with different doses of PGEO attenuated CAs induced by DOX in a dose-dependent manner. CA frequency decreased in the order of 32.0 ± 1.87, 23.60 ± 1.03, and 21.0±1.38 after administrations of 0.3, 0.6, and 0.9 mg/kg PGEO, respectively. The majority of aberrations induced by DOX were fragments and breaks. Robertsonian translocation (RT, Fig. 2c,d) appears in about 5% of examined metaphases. Multiple aberrations, more than three types of abnormalities in the same metaphase, were also recorded in 7.80% of the counted metaphases. A triradial configuration appeared in a few metaphases. Conversely, neither PGEO nor DOX (20 mg/kg) showed genotoxic effects on spermatocyte chromosomes. A dose of 40 mg/kg DOX induced a limited effect (Fig. 3).

Cell viability

PGEO was screened with six human cancer cell lines, including hepatocellular carcinoma (HepG2), prostate (PC3), lung carcinoma (A549), skin cancer (A431), colon cancer (HCT116), and breast cancer (MCF7) to explore anti-proliferation activity at concentrations of 100 µg/ml. PGEO has a highly cytotoxic effect with 100% death for all cells except breast cancer (MCF7) with 82.3% cells death with IC_{50} values of 6.2, 16.8, 13.3, 23.7, 25.2, and 61.9 µg/ml, respectively (Table 4).

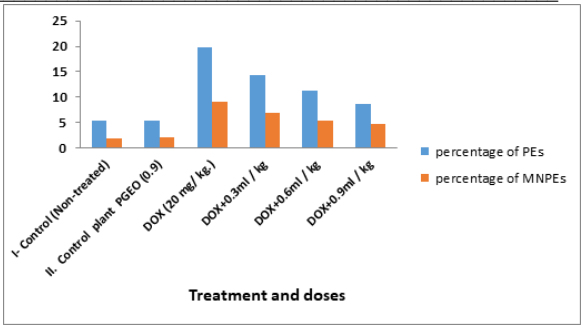

Figure 1. Mean percentage of polychromatic erythrocytes (PEs) and micronuclei (MNPEs) induced in mouse bone-marrow cells after treatment with doxorubicin (DOX) and *Pelargonium graveolens* essential oil (PGEO).

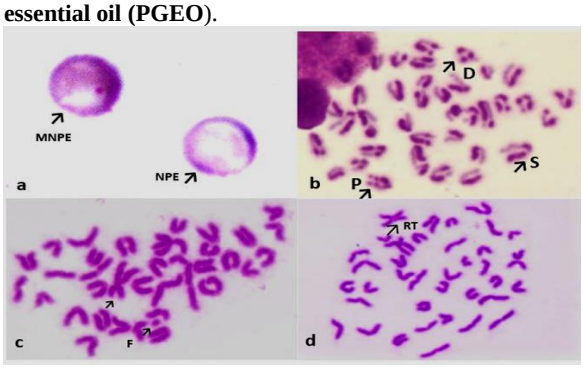

Figure 2. Plate in mice bone marrow cells showing (a) Micronuclei in polychromatic erythrocyte (MNPE) and normal PE, (b) Sister chromatid exchanges, S: Single, D: Double, P: Penta), (c) Chromosomal aberrations (Fragment and Robertsonian translocation), and (d) Robersonian translocation.

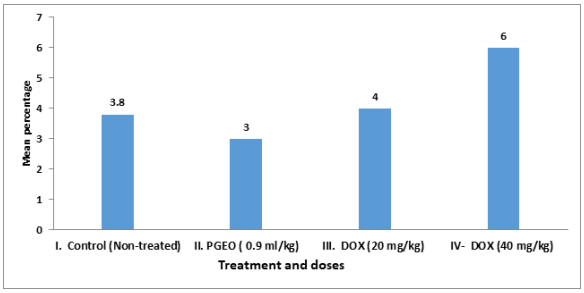

Figure 3. Mean percentage of metaphases with chromosomal abnormalities induced in mouse spermatocytes after treatment with doxorubicin (DOX) and *Pelargonium graveolens* essential oil (PGEO).

Discussion

Doxorubicin (DOX, Adriamycin) is an anthracycline antibiotic used as a chemotherapeutic drug for treating various adult and pediatric cancers *e.g.* breast cancer, Hodgkin's disease, or lymphoblastic leukemia [23]. DOX causes severe toxic side effects especially cardiotoxicity,myelosuppression and genotoxicity, due to it's no specificity in inducing cell death [24]. Plant extracts are frequently screened for new pharmacological compounds capable of protecting normal cells against cumulative toxicity of chemotherapy and radiotherapy [25].The essential oil

of *Pelargonium graveolens* (PGEO) was used to assess its impact on genotoxicity and the ability to attenuate the induced DNA damage by DOX was revealed. The study also demonstrated the anticancer effect of PGEO and characterized its chemical composition.

Table 1: Chemical composition of *Pelargonium graveolens* essential oil (PGEO) as indicated by GC-MS analysis

"No.	Compound	Rt	Concentration %
1	Linalool	17.31	4.97
2	Rose oxide	17.66	1.00
3	Cyclohexanone, 5-methyl-2-(1-methylethyl)-,cis	19.33	1.14
4	l-Menthone	19.77	7.26
5	α.-Terpineol	20.78	0.35
6	Citronellol	22.32	38.27
7	2-Octen-1-ol, 3,7-dimethyl-	22.59	1.38
8	Geraniol	23.18	12.39
9	6-Octen-1-ol, 3,7-dimethyl-, formate	23.73	10.23
10	Geranyl formate	24.58	2.94
11	Copaene	27.07	0.7
12	β-Bourbonene	27.39	1.65
13	Caryophyllene	28.50	1.83
14	6-Octen-1-ol, 3,7-dimethyl-, propanoate	29.06	0.21
15	ɣ-Muurolene	29.41	0.69
16	Humulene	29.61	0.52
17	Cedrene t	30.28	0.76
18	Germacrene-D	30.61	1.53
19	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	32.23	2.33
20	(3R,5aS,9aR)-2,2,5a,9-Tetramethyl-3,4,5,5a,6,7-hexahydro-2H-3,9a-methanobenzo[b]oxepine	33.33	0.33
21	Butanoic acid, 3,7-dimethyl-2,6-octadienyl ester, (E)	33.75	0.51
22	(+) Spathulenol	34.92	0.34
23	Caryophyllene oxide	35.15	0.29
24	Epicubenol	36.66	0.23
25	ɣ-eudesmol	36.99	7.14
26	Cubenol	37.19	0.2
27	Geranyl angelate	40.08	0.79

Rt: retention time

Table 2: Frequency of sister chromatid exchanges (SCE´s) /cell in mouse bone marrow after treatment with doxorubicin (DOX) and *Pelargonium graveolens* essential oil (PGEO).

Treatment and doses	SCE´s No	Mean ± S.E	SCE´s / cell Types*			Inhibitory index (%)
			Single	Double	Triple SCE and more	
I. Control (non-treated)	**514**	**4.11± 0.59 [a]**	**478**	**18**	**___**	**-**
II. PGEO (0.9 ml/kg)	**547**	**4.38± 0.48 [a]**	**483**	**32**	**___**	**-**
III. DOX (20 mg/ kg)	**4278**	**34.22±0.83 [e]**	**699**	**292**	**691**	**-**
IV -VI. DOX+PGEO						
+ 0.3ml/kg	**2410**	**19.28± 0.53 [d]**	**1165**	**262**	**197**	**49**
+ 0.6ml/kg	**2145**	**17.16± 0.37 [c]**	**1081**	**251**	**162**	**57**
+ 0.9 ml/kg	**1635**	**13.22± 0.59 [b]**	**955**	**190**	**99**	**70**

A total of 125 metaphases were analyzed (5 mice per group; 25 metaphase/mouse). One way ANOVA–Tukey's multiple comparisons test was used. The values having different superscript letters in each column are significantly different from one another. * Double = 2, Triple = 3 and so on.

The results of the present work revealed that DOX induced genotoxicity in somatic cells. A significant percentage of MNPEs and a notable toxicity in bone marrow cells was demonstrated after DOX treatment compared to the negative control. PGEO produced no effects on these parameters. Conversely, the frequency of micronuclei and degree of bone marrow toxicity in mice treated with DOX + PGEO decreased significantly compared to animals receiving DOX alone. Thus, PGEO appears to display anti-genotoxic activity. Venkatesh et al. [26] and Boriollo et al. [27] also demonstrated that DOX caused bone marrow toxicity and MNPEs. Also, a statistically significant dose-dependent increase in micronucleus frequency in cultured human lymphocytes treated with DOX *in vitro* was detected by Dhawan et al. [28]. The cytotoxic effect of DOX may be related to the inhibition of nuclear topoisomerase II [29].

Table 3: Frequency of chromosomal aberrations induced in mouse bone-marrow cells after treatment with doxorubicin (DOX) and *Pelargonium graveolens* essential oil (PGEO).

Treatment and doses	Total abnormal metaphases		No and (%) of metaphases with different types of chromosomal aberrations					Inhibitory index (%)
	No	Mean(%) ± SE	Chromatid and/or chromosome gap	Fragment and/or Break	Multiple aberrations	RT+ break / Fragment	Endomitosis	
I. Control (Non treated)	11	2.20 ± 0. 37[a]	10(2.0)	1(0.20)	-	-	-	-
II.PGEO (0.9ml/kg)	13	2.60 ± 0. 24[a]	10(2.0)	3(0.60)	-	-	-	-
III. DOX (20mg/kg)	201	40.20 ± 1. 73[d]	1(0.20)	129(25.80)	39(7.80)	25(5.0)	7(1.40)	-
IV-VI. DOX + PGEO								
+0.3 ml/kg	160	32.0 ± 1. 87[c]	3(0.60)	116(23.20)	23(4.60)	18(3.60)	-	22
+0.6 ml/kg	118	23.60 ± 1. 03[b]	1(0.20)	82(16.40)	14(2.80)	21(4.20)	-	44
+0.9ml/kg	105	21.0 ± 1. 38[b]	2(0.40)	75(15.0)	11(2.20)	17(3.40)	-	51

Harvest time 24h after the last treatment, Number of examined metaphases = 500 (100 metaphase/animal, 5 animals/group). One way ANOVA–Tukey's multiple comparisons test was used. RT: Robertsonian translocation. The values having different superscript letters in each column are significantly different from one another. The data were presented as mean ± SE (n= 5).

Table 4: Percent activity of *Pelargonium graveolens* essential oil (PGEO) at 100 µg/ ml and IC_{50} on different cell lines.

HEPg2		PC3		A549		A431		HCT116		MCF7	
100µg/ ml	IC_{50} µg/ ml	100µg/ml	IC_{50} µg/ml	100µg/ml	IC_{50} µg/ ml	100µg/ ml	IC_{50} µg/ ml	100µg/ ml	IC_{50} µg/ ml	100µg/ml	IC_{50} µg/ ml
100%	6.2	100%	16.8	100%	13.3	100%	23.7	100%	25.2	82.3	61.9

SCE is a standard assay for assessing genotoxicity and is a biomarker of exposure to mutagens/carcinogens. The results of the present work revealed that, DOX is a potent inducer of SCEs in bone marrow cells. The frequency of SCEs/cell was 34.22 ± 0.83 after treatment with DOX at 20 mg/kg, almost eight times basal levels. A pronounced number of chromosomes with extensive SCE was recorded after DOX treatment. PGEO pretreatment significantly ameliorated DOX-induced SCE by 49%, 57%, and 70% at different concentrations, that is, 0.3, 0.6, and 0.9 ml/kg, respectively. Thus, this oil shows anti-genotoxic and anti-oxidative potential. The high incidence of SCEs induced after treatment with DOX may reflect its action on cellular DNA processes [30]. Our results are similar to those of Jenkhetkana et al. [31], who reported that DOX is a strong inducer of SCEs in human lymphocytes *in vitro*.

In the present study, a highly significant percentage of CAs was observed in bone marrow cells after DOX treatment. Many aberrations were structural, including fragments or breaks. These CAs were found in 25.80% of counted metaphases. Robertsonian translocation (RT, 7.80%) and metaphases containing multiple aberrations were notable. A triradial configuration appeared in a few metaphases from mice treated with DOX or DOX + PGEO. Previously, Anderson et al. [32] demonstrated an increase in DNA strand breaks and a significant percentage of chromosomal anomalies in FISH and conventional chromosomal aberration assays after DOX administration. RTs or centric fusions are among the most common types of rearrangements. RTs are a specific class of translocations in which two acrocentric chromosomes fuse at their centric ends. In humans, this type of aberration is associated with some disorders because of mal-segregation of the translocated chromosome. Hajra et al. [9] also reported that DOX (5 mg, i.p.) induced chromosomal aberrations, micronuclei formation, DNA damage, and apoptosis in bone marrow. The present study demonstrated that PGEO mitigated DOX-induced chromosomal abnormalities by about 22%, 44%, and 51%, as doses increased. Protection against DOX-induced genotoxicity by PGEO may be due to free radical scavenging and better antioxidant status.

In contrast, DOX, at the dose of 20 mg/kg, induced no significant number of chromosomal abnormalities in spermatocytes (germ cells). A dose of 40 mg/kg DOX caused a limited increase in CAs compared to control. Genetic damage to germ cell chromosomes is indirectly connected with transmissible genetic damage. This effect of DOX is a point of interest for using the drug in chemotherapeutic regimens. The same effect is noticed for cisplatin [25]. These data for DOX are useful for comparisons with chemotherapeutic drugs that substantially affect germ cell chromosomes, e.g., etoposide, methotrexate, tamoxifen, vinblastine, and 5-florouracil [33-35, 25].

The present results demonstrated that DOX is a powerful inducer of genotoxicity in somatic cells. DOX-induced genotoxicity is due to direct binding to DNA *via* intercalation between base pairs, and inhibition of DNA repair *via* blocking topoisomerase II. These actions result in a blockade of DNA and RNA synthesis and fragmentation of DNA. DOX is also a powerful iron chelator. The iron–DOX complex can bind DNA and cell membranes, producing free radicals that immediately cleave DNA and disrupt membranes [36].

As previously mentioned, DOX acts by inducing large amounts of ROS to kill cancer cells, but it may also affect normal cells. Many pathological conditions display an imbalance in redox potential when excessive amounts of ROS cannot be removed. ROS is well documented to cause damage to cellular macromolecules and likely underlies many human diseases. The protective role of PGEO evidenced in this study may be attributed to its strong antioxidant, anticancer, and anti-inflammatory properties [37, 38, 5]. Further, PGEO has a highly cytotoxic effect on all human cancer cells lines examined in this studyincluding hepatocellular carcinoma (HepG2), prostate cancer (PC3), lung carcinoma (A549), skin cancer (A431), colon cancer (HCT116), and breast cancer (MCF7), with ~100% cells death at a concentration of 100 µg/ml.. Gautam et al. [39] reported that multiple pathways and mechanisms underlie the anticancer and anti-mutagenic effects of plant essential oils, including DNA repairmodulation, regulation of tumor suppressor proteins (p53 and Akt), transcription factors (NF-*κ*B and AP-1), MAPK pathway, and detoxification enzymes, such as SOD, catalase, glutathione peroxidase, and glutathione reductase. Stringaro et al. [3] reported that free radicals generated by damaged membranes, when combined with essential oils, produce radicals with scavenging activities. Another benefit of PGEO in the present work is that it did not display mutagenic activity by its own and shows relatively low toxicity.

The present study also characterized the chemical composition of PGEO. The oil isolated by hydro-distillation was analyzed *via* GC/MS. The most biologically active compounds in geranium oil are citronellol (CT, 38.27%), geraniol (12.39%), 1-menthone (7.26 %), and linalool (4.97%). Rao et al. [40] and Fayed [37] also reported that geranium essential oil is rich in oxygenated components and rhodinol (CT + geraniol + linalool).

CT in PGEO is a monoterpene predominantly formed by plant secondary metabolism. The antioxidant activity of CT was studied using the DPPH and FRAP methods [41]. The authors reported that CT is a strong antioxidant and demonstrated good radical scavenging activity (86%) as compared to ascorbic acid (96 %) and about 50% of the antioxidant activity as compared with trolox in the FRAP assay. Melo et al. [42] reported that CT is of potential benefit for managing inflammatory disorders and associated damage caused by oxidant agents.

Geraniol (12.39%) is the second major constituent of PGEO. Geraniol is a natural monoterpene known for its wide spectrum of pharmacological activities, *e.g.* anti-inflammatory, antioxidant, neuroprotective, anticancer, and stimulation of anti-oxidative defenses [43]. It might serve as a therapeutic agent for the treatment of many cancers, including lung, pancreatic, hepatic, and kidney [44]. The activity of geraniol has major clinical implications; this PGEO component is "generally recognized as safe" (GRAS) [45]. Also menthone was reported to have strong antioxidant capacity [46, 47].

Linalool, a monoterpene alcohol, contributes 4.97% to PGEO constituents. Linalool displays several biological activities, including analgesic, anti-inflammatory, and antioxidant. Anti-inflammatory effects of linalool are associated with the modulation of pro-inflammatory cytokines and antioxidant enzymes. Importantly, linalool reduced the levels of nuclear factor erythroid 2, a regulator of antioxidant stress [48]. Linalool was effective as an antioxidant in guinea pig brains injected with H_2O_2 [49], a reagent used in antioxidant studies. Linalool decreased oxidative stress in male Wister rats by modulating malondialdehyde, a marker for lipid peroxidation, and increasing glutathione content [50]. Iwasaki et al. [51] demonstrated that linalool showed anticancer effects *via* cancer-specific oxidative stress and documented its application in colon cancer therapy. Anticancer potential of linalool was also recorded against solid tumor cell lines, such as gastric cancer, lung cancer, skin cancer [52], and hepatic cancer (HepG2) [53], as well as several leukemia cell lines [54]. PGEO is a mixture of many active constituents that all together contribute to its protective role as demonstrated by the evaluation of several mutagenic, and cancer cell assay endpoints.

Conclusion

Research has recently focused on the safety and possible new activities of herbal products. This advance is welcome and may lead to the development of new and beneficial agents for human health. The focus of the present study, PGEO, is a promising source of natural products with notable anti-genotoxic and anti-carcinogenic activity. PGEO may be useful in combination with chemotherapy for reducing their serious side effects.

Conflict of interest

The authors would like to declare that there are no conflict of interest.

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