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Eucalyptus globulus Essential Oil modulates Genotoxicity and Histological Alterations Induced by Mitomycin C in Mice

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Abstract

Recent findings indicated that plant-based diet minimizes the overall cancer risk. Accordingly, there is a great concern with the therapeutic and anticancer uses of plant essential oils. The current work aimed to evaluate the protective activity of *Eucalyptus globulus* essential oil (EGEO) against genotoxic and histopathological defects induced by mitomycin C (MMC) in mice. Sixty male mice were subdivided into six groups: I: Control negative; II: Treated with 1.2 mL EGEO/kg for 4 days; III: MMC (1.5 mg/kg); IV-VI: three combined groups treated with EGEO at (0.8, 1.0 or1.2 mL/kg) plus MMC at (1.5 mg/kg). Twenty-one compounds, with the superiority of eucalyptol (74.31%), were determined in EGEO using Gas chromatography–mass spectrometry (GC/MS) analysis. Treatment with MMC triggered substantial genotoxic and histopathological lesions in mouse tissues. MMC induced defects were significantly reversed by EGEO treatment at the tested doses. Chromosomal aberrations (Cas), micro-nucleated polychromatic erythrocytes (MNPCEs), comet tail formation, inflammatory cell infiltration, necrosis, Kupffer cells, and pyknotic cells were all relevantly diminished. In conclusion, EGEO could be a safe and promising candidate for pharmaceutical and food industries. The synergistic effects of EGEO active constituents could alleviate the chemotherapeutic agents MMC-induced genetic and histological defects, thereby improving biological activities.

***Keywords*:** *Eucalyptus globulus* Essential oil, GC/MS analysis, Mitomycin C; Chromosomal aberrations; Micro-nucleated polychromatic erythrocytes.

1. Introduction

Mitomycin C (MMC) is a natural antibiotic initially isolated from *Streptomyces caespitosus*, which was proven to have antitumor and growth inhibitor activities [1]. MMC is widely used to manage various types of solid tumours: gastric carcinoma, superficial urinary bladder cancer, pancreatic cancer, anal and oesophageal carcinomas. MMC is also used to treat the problems associated with tissue transplantation and hematologic malignancies [2]. The synergistic effect of MMC with radiotherapy assists in targeting hypoxic cells in radiation-resistant tumours [3]. MMC was reported to be a genotoxic and cytotoxic agent by inducing DNA lesions (adducts), DNA breaks, chromosomal aberrations, and micronuclei formation [1, 2, 4, 5].

Recent studies aim to produce antitumor agents that exert maximum effective inhibition to proliferating cancer cells with minimum side effects or to explore natural products that reduce the side effects of chemotherapy. Currently, essential oils (EOs)-derived from plants have a growing importance in folk and medicinal applications. Many studies have demonstrated various biological activities of EOs, such as the antioxidant, antiviral, antibacterial, insecticidal, anti-mutagenic, and anti-carcinogenic effects [6, 7]. Many volatile oils have a promising role in treating various human diseases,

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and their fragrances are used for improving mood and as depression release. Some EOs have been used in aromatherapy, food preservation, perfumery, and cosmetics industries [8].

Among the various botanical EOs recorded in commercial applications are EOs from *the Eucalyptus* species. *Eucalyptus* represents one of the most planted genera in the world. Many EOs from Eucalyptus species are rich in monoterpenes and have many biological and applications organoleptic properties [9]. *Eucalyptus globulus* (EG) is considered one of the most substantial members of the *Myrtaceae* family, which comprises about 900 species. Fewer than 20 of these species contain more than 70% of eucalyptol (1,8 cineole) in their EOs which is responsible for giving the oil its distinctive eucalyptus aroma. *Eucalyptus globulus* essential oil (EGEO) is most well-known for its considerable benefits for respiratory applications: colds, flu, fever, sinusitis and bronchitis. EGEO is used in infectious diseases such as urinary infections, cystitis, parasitic infections, pain relief, muscular aches, arthritis, and rheumatism. Recent studies have proved that EOs of *Eucalyptus have been used* as an effective therapy against the Covid-19 virus [10]. Several studies have reported Geno-protective properties of some individual monoterpenes such as linalool, myrcene, eucalyptol, camphor, Limonene, and thujone in vitro system using bacteria and cultured mammalian systems [11-14]. However, no previous study explored the genotoxicity and antigenotoxicity of EGEO in mouse bone marrow cells. Therefore, the current investigation evaluated the following aims: (1) Determination of the chemical texture of the EGEO using gas chromatography-mass spectrometry GC/MS; (2) Examination of genotoxic and Geno-protective properties of EGEO in mouse bone marrow cells using cytogenetic techniques and histopathological examination.

2. Material and Methods

2.1. Plant material and extraction of EO

The EG leaves were gathered *f*rom the Experimental Agricultural Station, Faculty of Agriculture, Cairo University, Cairo Governorate. Plant leaves were used for the determination of volatile oil content. The volatile oil of EG leaves was extracted by the hydro-distillation method in a Clevenger's apparatus [15]. The oily layer was separated and dried with anhydrous sodium sulfate (0.5 g). EGEO was reserved in sealed airtight glass flasks and enclosed with aluminum foil at 4^{o}C until analysis. The sample was done in triplicate and the mean values of the oil content (percent) were registered. The EG leaves contain 1.13 % essential oil.

2.2. Gas chromatography–mass spectrometry analysis (GC-MS)

Analysis of EGEO was applied by the GC-MS technique using the GC-MS instrument (THERMO Scientific ™ Corporate, Waltham, MA, USA). The GC-MS instrument was adjusted according to the subsequent settings: TRACE GC Ultra Gas Chromatographs lined with a Thermo Scientific ISQ™ EC single quadrupole mass spectrometer. The GC-MS system was equipped with a TR-5 MS column with dimensions of 30 m × 0.32 mm, i.d., 0.25 µm film thickness. Helium was used as carrier gas at a flow rate of 1.0 mL/min with a split ratio of 1:10 using the next temperature program: 60 ◦C for 1 min then rising at 4.0 ◦C/min to 240 ◦C, and held for 1 min. The injector and detector were held at 210 ◦C. An aliquot of 1 µL of diluted samples in hexane (1:10, v/v) was always injected. Mass spectra were registered by electron ionization (EI) at 70 eV, using a spectral range of m/z 40–450. The chemical constituents of the EGEO were distinguished by Automated Mass spectral Deconvolution and Identification (AMDIS) software, version 2.71 (Gaithersburg, MD, USA) (www.amdis.net), retention indices (relative to n-alkanes C8–C22), and comparison of the mass spectrum with authentic compounds (if available) from the Wiley spectral library collection and NIST library database (Gaithersburg, MD, USA; Wiley, Hoboken, NJ, USA).

2.3. Experimental animals

This study was carried out using 9-12 weeks-old Swiss male mice weighing approximately 25g purchased from Animal House Colony, National Research Centre. Mice were housed in filter-top polycarbonate cages in an air-conditioned room free from any source of chemical contamination at temperature (25 ± 1°C) with a 12-hour light/dark cycle. Mice were provided with a standard lab diet (protein: 160.4; fat: 36.3; fiber: 41 g/kg and metabolizable energy 12.08 MJ, purchased from Meladco Feed Co., Aubor City, Cairo, Egypt). All animals received humane care in compliance with the guidelines of the Animal Care and Use Committee of the National Research Canter, Dokki, Cairo, Egypt (Approval # NRC-11010345) and the National Research Council's Guide for the Care and Use of Laboratory Animals and the National Institutes of Health (NIH publication 86-23 revised 1985).

2.4. Experimental design

Sixty animals were randomly divided into six equal groups (10 mice/group) as follows:

- **Group 1**: Non-treated negative control
- **Group 2**: Mice were orally administrated 1.2 mL/kg of EGEO for four successive days. Such concentration was selected based on studies of Gebremickael [16].
- **Groups 3:** Mice were intraperitoneally injected with 1.5 mg/kg of MMC for 24 h (Sigma-Aldrich Chemie GmbH, Germany, Y0000378). This dose was selected based on previous genotoxic studies in mouse bone marrow cells [1, 17].
- **Groups 4-6:** Three groups were orally given three concentrations of EGEO (0.8, 1 and 1.2 mL/kg, for four days) plus a single intraperitoneal dose of MMC (1.5 mg/kg) 24 h. before sacrifice. Five animals from each group were injected with colchicine (3.0 mg/kg) 2 h before sacrifice, and bone marrow cells were collected for chromosomal aberrations analysis. Bone marrow, liver, and kidney were taken from the other half of the animals in each group for micronucleus, comet assay and histopathological investigation.

2.5. Chromosomal aberration (CAs) assay

Bone marrow chromosomes were prepared as described earlier [7]. Briefly, both femurs were used to collect bone marrow cells which were incubated in hypotonic solution (0.075 M KCL) for 20 min at 37°C. The cell suspension was centrifuged, and the pellets were fixed in methanol/glacial acetic acid (3:1). Finally, cells were spread onto slides, and stained with Giemsa. One hundred metaphases were analysed per animal (5 mice/ group) using a light microscope at 1500× magnification.

2.6. Micronucleus (MN) assay

The The micronucleus preparation from bone marrow was performed as described previously in detail [7]. Bone marrow cells were gathered from femurs in fetal bovine serum, centrifuged, and smeared on clean slides, air-dried and fixed in absolute methanol. Then, the slides were stained with the May Grünwald-Giemsa protocol and analysed using a light microscope at 1000× magnification. MN was recognized as dark blue bodies in the cytoplasm of polychromatic erythrocytes (PCEs). One thousand PCEs were scored per mouse (5 animals/group) to determine the frequency of micronucleated polychromatic erythrocytes (MNPCE).

2.7. Single cell gel electrophoresis (comet assay)

Alkaline comet assay was performed using mouse bone marrow cells as represented formerly in detail. [7] The bone marrow cells were suspended in 0.8% agarose (low-melting-point) and spread onto full-forested slides pre-coated with 1% standard agarose and refrigerated at 4°C in the dark for 5 min to solidify on the agarose. The slides were kept in lysis buffer (10 mM Tris-HCl, 100 mM EDTA, 2.5 M NaCl, 1% Triton X-100, 10% DMSO, pH 10) at 4°C for 2 h. The slides were maintained in ice-cold alkaline buffer (300 mM NaOH, 1 mM EDTA, pH 13) for 30 min, followed by electrophoresis at 300 mA for 30 min then rinsed three times in neutralization buffer (400 mM Tris-HCl pH 7.5) and fixed with ethanol (70%) for 5 min each. The dried slides were stained with ethidium bromide and promptly examined at 400× magnification with a fluorescent microscope. Two hundred nucleoids were analysed per animal using computerized Comet score™ software (TriTek Corp, version 2.0.0.0, Sumerduck, VA 22742, United States). The percentage of DNA in the comet tail (% tail DNA) and Olive tail moment (OTM) are used to quantify DNA damage. The values of OTM are expressed in arbitrary units (A.U) and the cells with > 80% DNA in the tail region were classified as hedgehogs and excluded from software analysis**.**

2.8. Histopathological studies

Pieces of liver and kidney were fixed in neutral buffered formalin (10%) for 24 hours then the samples have embedded in paraffin wax, sectioned at 5 μm and stained with hematoxylin and eosin (H & E).

2.9. Data analysis

Data were analysed employing SPSS software (Statistical Package of Social Science, version 20, Armonk, New York: IBM Corp). The data were checked for normality and the homogeneity of the variance using the Kolmogorov-Smirnov's test and Levene's test, respectively. The differences among groups with normal distribution were analysed by one-way analysis of variance (ANOVA) followed by Turkey's honestly significant difference test (Turkey's HSD). The results were regarded as significant when the P-value was less than 0.05.

3. Results

3.1. Identification of chemical composition of EGEO by GC/MS analysis

As depicted in Table (1), EGEO possessed four main chemical groups: (1) oxygenated monoterpenes represented 76.25 % of the oil concentration with the superiority of eucalyptol (74.31%). (2) monoterpene hydrocarbons (14.34%) in which cymene, D-limonene, α-pinene and γ-terpinene represent 6.81%, 3.67%, 2.12 and 1.55%, respectively; (3) oxygenated sesquiterpenes represented 7.39% of the oil concentration in which globulol represented 4.06%;

and (4) sesquiterpenes hydrocarbons (1.58%) in which aromadendrene represents 1.20%.

Table (1): The EGEO composition as indicated by GC-MS of analysis

No	Compound Name	RT	KI_{Exp}	KI_{Lit}	Area %	Identification
Monoterpene hydrocarbons					14.34	
1	*α*-Pinene	3.89	940	939	2.12	MS, KI
2	*β*-Pinene	4.84	982	980	0.19	MS, KI
3	Cymene	6.04	990	991	6.81	MS, KI
4	D-Limonene	6.10	1033	1031	3.67	MS, KI
5	γ-Terpinene	6.90	1052	1054	1.55	MS, KI
Oxygenated monoterpenes					76.25	
6	Eucalyptol	6.22	1034	1033	74.31	MS, KI
7	Isoamylvalerate	8.31	1096	1095	0.18	MS, KI
8	L-Pinocarveol	9.48	1133	1135	0.58	MS, KI
9	Terpinen-4-ol	10.79	1175	1174	0.59	MS, KI
10	cis-p-Mentha-1(7),8-dien-2-ol	11.08	1177	1178	0.26	MS, KI
11	*α*-Terpineol	21.86	1190	1186	0.33	MS, KI
Sesquiterpene hydrocarbons					1.58	
12	Aromadendrene	18.78	1435	1439	1.20	MS, KI
13	Alloaromadendrene	19.46	1453	1458	0.38	MS, KI
Oxygenated sesquiterpenes					7.39	
14	Epiglobulol	22.69	1533	1532	0.60	MS, KI
15	Palustrol	22.9	1564	1567	0.30	MS, KI
16	(-)-Spathulenol	23.16	1575	1577	0.91	MS, KI
17	Globulol	23.39	1591	1590	4.06	MS, KI
18	Viridiflorol	23.65	1595	1592	0.72	MS, KI
19	Guaiol	23.74	1601	1600	0.18	MS, KI
20	Ledol	23.95	1604	1602	0.17	MS, KI
21	β-Eudesmol	24.54	1645	1649	0.45	MS, KI
	Total				99.58	

Rt: Retention time; KI_{Lit}: Kovats retention index on a DB-5 column in reference to n-alkanes; KI_{Exp}: Experimental Kovats retention index. Identification of EO components was drtermined upon the mass spectral data (MS) and Kovats indices (RI) with those of Wiley spectral library collection and NIST library databases

3.2. Effect of EGEO and MMC on the induction of CAs

As shown in Table 2 and Figure 1, treatment with a single dose of MMC (1.5 mg/kg, for 24 h) statistically increased the percentage of CAs (41.60%) in mouse bone marrow cells compared to the control group. Oral administration of EGEO (1.2 mL/kg for four days) did not statistically increase the occurrence of the CAs (4.40%) compared to the control group (4.0 %). Three concentrations of EGEO significantly reduced the MMC-induced frequency of CAs (34.60%, 24.20 % and 20.60%, respectively) compared to the group treated with MMC only (41.60%) in a concentration-dependent pattern.

Table (2): Effectiveness of the treatment with EGEO on MMC-induced chromosomal aberrations in mouse bone marrow cells

Treatment and doses	Total abnormal metaphases		The no and (percentage) of metaphases with various types of chromosome aberrations			
	No.	Mean% ± S. E	Gap	Fr and/or Br	MA	RT
Negative Control	20	4.00 ± 0.32[a]	4(0.80)	16(3.20)	-	-
Plant Control (EGEO,1.2 mL/kg)	22	4.40 ± 0.40[a]	6(1.20)	16(3.20)	-	-
Positive control MMC						
MMC (1.5 mg/ kg)	208	41.60 ± 0.94[c]	3(0.60)	63(12.60)	139(27.80)	3(0.60)
Treatment combination						
MMC (1.5mg/kg) + EGEO (0.8 mL/kg)	173	34.60 ±0.51[b]	4(0.80)	59(11.80)	108(21.60)	2(0.40)
MMC (1.5mg/kg) + EGEO(1mL/kg)	121	24.20± 1.32[b]	5(1.0)	73(14.60)	43(8.60)	-
MMC (1.5mg/kg) + EGEO(1.2mL/kg)	103	20.60 ±1.63[b]	8(1.60)	65(13.0)	30(6.0)	-

No. of examined cells = 500 cells/ group (100 cells/ mouse).
RT: Robertsonian translocation. Fr and/or Br: Fragment and/or Break. MA: Multiple aberrations.
The values with different superscript letters in each column are statistically significantly different from one another as calculated by One-way ANOVA followed by Tukey HSD test

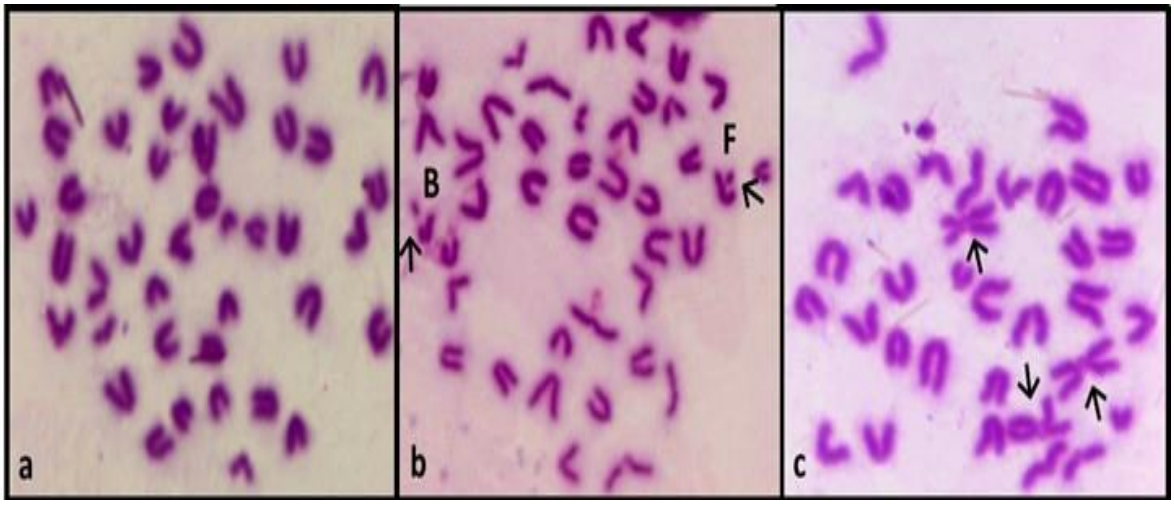

Figure (1): Light microscope photomicrographs showing chromosomal abnormalities in mouse bone marrow cells: (a) normal, (b) fragment and break, and (c) Robertsonian translocation (RT) (original magnification 1500×)

3.3. Effect of EGEO and MMC on the induction of MNPCEs

As shown in Table 3 and Figure 2, the percentage of MNPCEs in bone marrow cells statistically did not increase after oral administration of EGEO at a concentration of 1.2 mL/kg (3.00%, for four days) compared to the negative control group (2.26%). By contrast, the level of MNPCEs statistically increased after treatment with three tested doses of MMC (19.00%, 28.20% and 55.80 %) compared to the negative control. Pre-administration of three concentrations of EGEO (0.8, 1.0 and 1.2 mL/kg) significantly reduced MMC-increased MNPCEs (22.80%, 20.60%, and 19.40%, respectively) compared to the MMC-treated group (28.20%)

3.4. Effect of EGEO and MMC on the induction of comet tail formation

In comparison with the control group, treatment with MMC (1.5 mg/kg) caused a considerable rise in the percentage of tail DNA (10.90%) as shown in Table 3 and Figure 3. Four days of repeated treatment with EGEO (1.2 mL/kg) did not cause a statistical elevation in the proportion of tail DNA (6.29%). Interestingly, three concentrations of EGEO (0.8, 1.0, 1.2 mL/kg) effectively reversed the genotoxic effect of MMC through a significant decrease in the percentage of tail DNA (7.69%, 7.82%, and 6.85%, respectively) compared to MMC- treated group. The values of OTM were also decreased in the co-treated groups with EGEO and MMC (1.54, 1.34, and 1.02 A. U, respectively) compared to MMC-treated group (1.72 A. U).

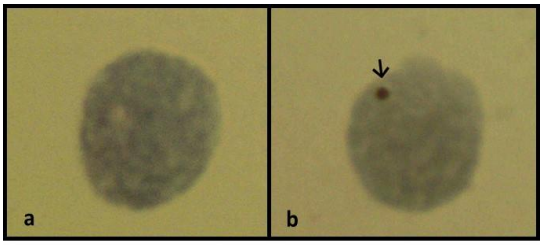

Figure (2): Light microscope photomicrographs from mouse bone marrow showing: (a) PCE, and (b) MNPCE (original magnification 1000×)

Table (3): Effectiveness of the treatment with EGEO on MMC-induced micronuclei and comet tail formation in mouse bone marrow cells

Treatment and doses	Micronuclei formation		Comet tail formation	
	No.	MNPCEs (%)	Tail DNA (%)	OTM (A.U)
Negative Control	113	2.26 ± 0.37^{a}	5.02 ± 0.39^{a}	0.74 ± 0.02^{a}
Plant Control (EGEO,1.2 mL/kg)	151	3.00 ± 0.45^{a}	6.29 ± 0.16^{ab}	0.84 ± 0.04^{ab}
Positive control MMC				
MMC (1.5 mg/ kg)	1413	28.20 ± 1.07^{c}	10.90 ± 0.79^{d}	1.72 ± 0.26^{de}
Treatment combination				
MMC (1.5 mg/kg) + EGEO (0.8 mL/kg)	1142	22.80 ± 0.86^{b}	7.69 ± 0.79^{bc}	1.54 ± 0.26^{bcd}
MMC (1.5 mg/kg) + EGEO (1mL/kg)	1033	20.60 ± 0.81^{b}	7.82 ± 0.22^{bc}	1.02 ± 0.01^{bcd}
MMC (1.5 mg/kg) + EGEO (1.2 mL/kg)	974	19.40 ± 0.93^{b}	6.85 ± 0.36^{b}	1.34 ± 0.08^{bcd}

Data expressed as Mean % ± S.E.
10000 cells per treatment (5 mice/group) were scored for micronucleus and comet assays, respectively. The values with different superscript letters in each column are statistically significantly different from one another as calculated by One-way ANOVA followed by Tukey HSD test

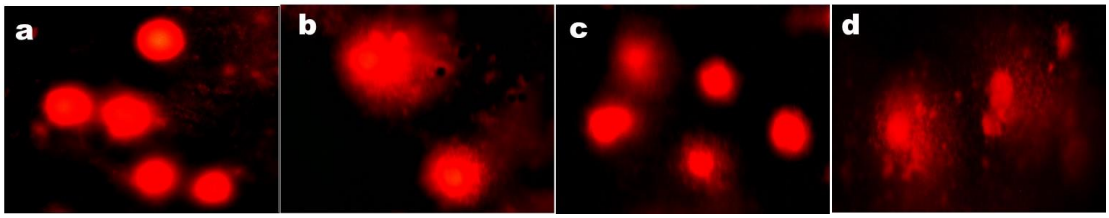

Figure (3): Fluorescent microscope photomicrographs from mouse bone marrow cells showing DNA damage measured by alkaline comet assay (a) intact cells; (b,c,d) different pattern of comet tail formation (magnification 400×)

3.5. . Effect of EGEO and MMC on liver histopathological sections

The liver sections of negative control and plant control groups (1.2 mL/kg) exhibited a normal structure of hepatocytes radiated from the central vein and separated from each other by blood sinusoids with normal spherical nuclei (Figure 4A and B). On the other hand, MMC-treated mice showed severe pathological distortions: disruption in hepatic structure, congestion of central vein, accumulation of a large number of inflammatory cells, necrotic lesion areas, Pyknotic nuclei, and proliferation of Kupffer cells (Figure 4C). In the combined groups, EGEO ameliorated such histological alterations in a concentration-dependent manner. The liver sections from two co-treated groups with MMC and EGEO (0.8 and 1 mL/kg) exhibited hepatic lobules with mild inflammatory cells, few pyknotic nuclei, and Kupffer cells (Figure 4 D &E). The liver sections of the co-treated group with MMC and EGEO (1.2 mL/kg) showed a nearly normal structure with minimal pyknotic nuclei and Kupffer cells (Figure 4 F).

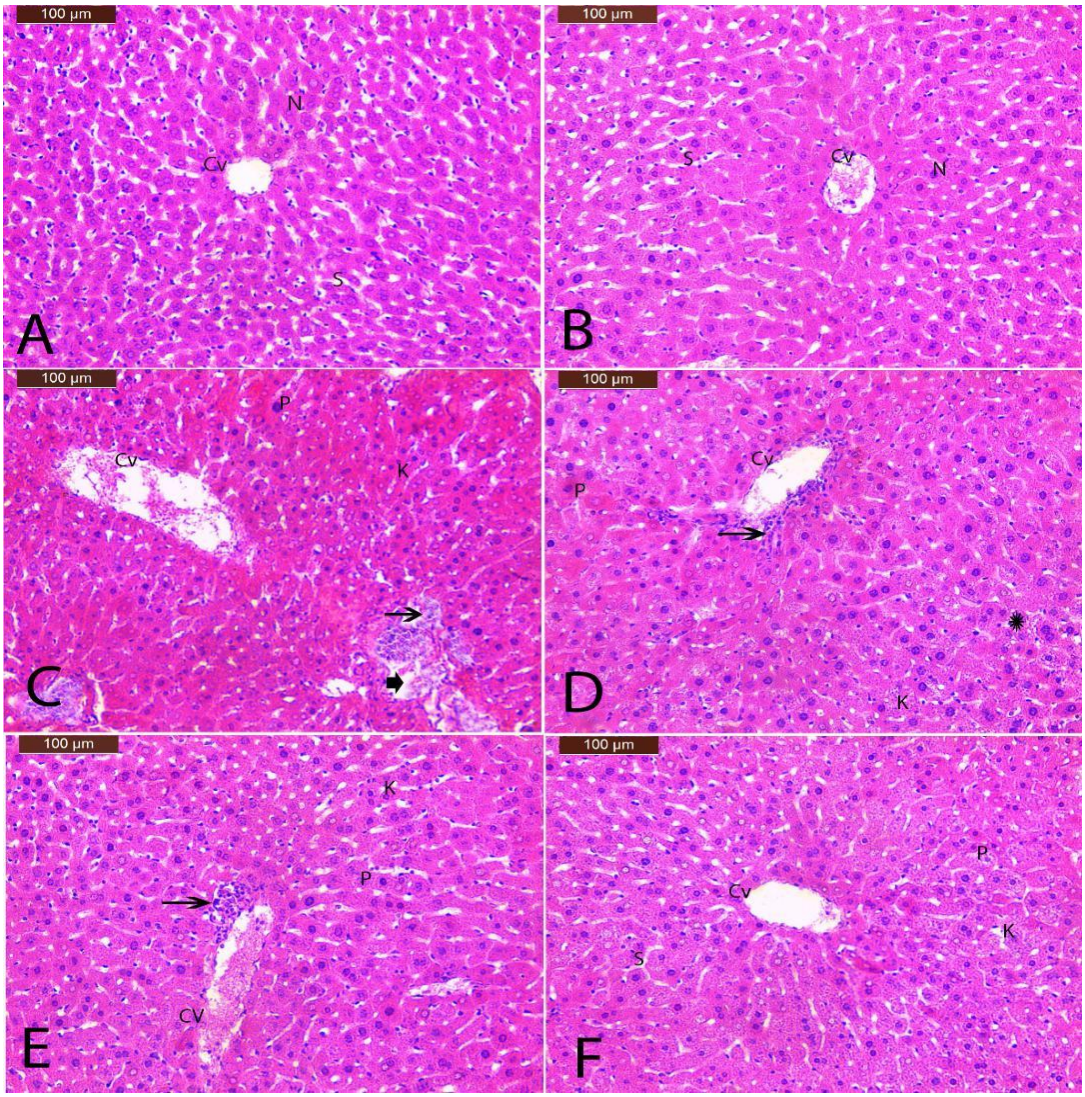

Figure (4) Representative H & E-stained liver sections showing experimental groups. A: Negative control; B: EGEO-treated group (1.2 mL/kg); C: MMC-treated group; D, E, and F: three co-treated groups with MMC and EGEO at three concentrations 0.8, 1 and 1.2 mL/kg, respectively. Central vein (CV), blood sinusoids (S), nucleus (N), pyknotic nuclei (P), Kupffer cells (K), inflammatory cells (arrow), necrotic lesion area (arrowhead or star)

3.6. Effect of EGEO and MMC on kidney histopathological sections

The sections from kidneys from control and EGEO (1.2 mL/kg) exhibited typical structure of glomeruli, regular Bowman's space, and renal tubules, which included distal convoluted tubules and proximal convoluted tubule (Figure 5 A &B). On the other hand, MMC significantly caused kidney histopathological lesions: degeneration of the glomerular and tubules architecture, dilation of Bowman's space; infiltration of inflammatory cells; interstitial haemorrhage; necrotic changes and pyknotic nuclei in many proximal and distal convoluted tubule (Figure 5C). These renal lesions were restored in three combined-treated groups. The kidney sections from co-treated group with MMC and EGEO (0.8 mL/kg, Figure 5D) showed moderate amelioration of kidney structure with mild dilated Bowman's spaces, mild infiltrated of inflammatory cells, necrotic area, and pyknotic nuclei in some proximal and distal convoluted tubules. Kidney sections from the co-treated group with MMC and EGEO (1 and 1.2 mL/kg, Figure 5 E & F, respectively) exhibited more or less typical structure with mild dilated Bowman's space, minimal tissue necrosis, and few pyknotic nuclei in few proximal and distal convoluted tubules (Figure 5E & F).

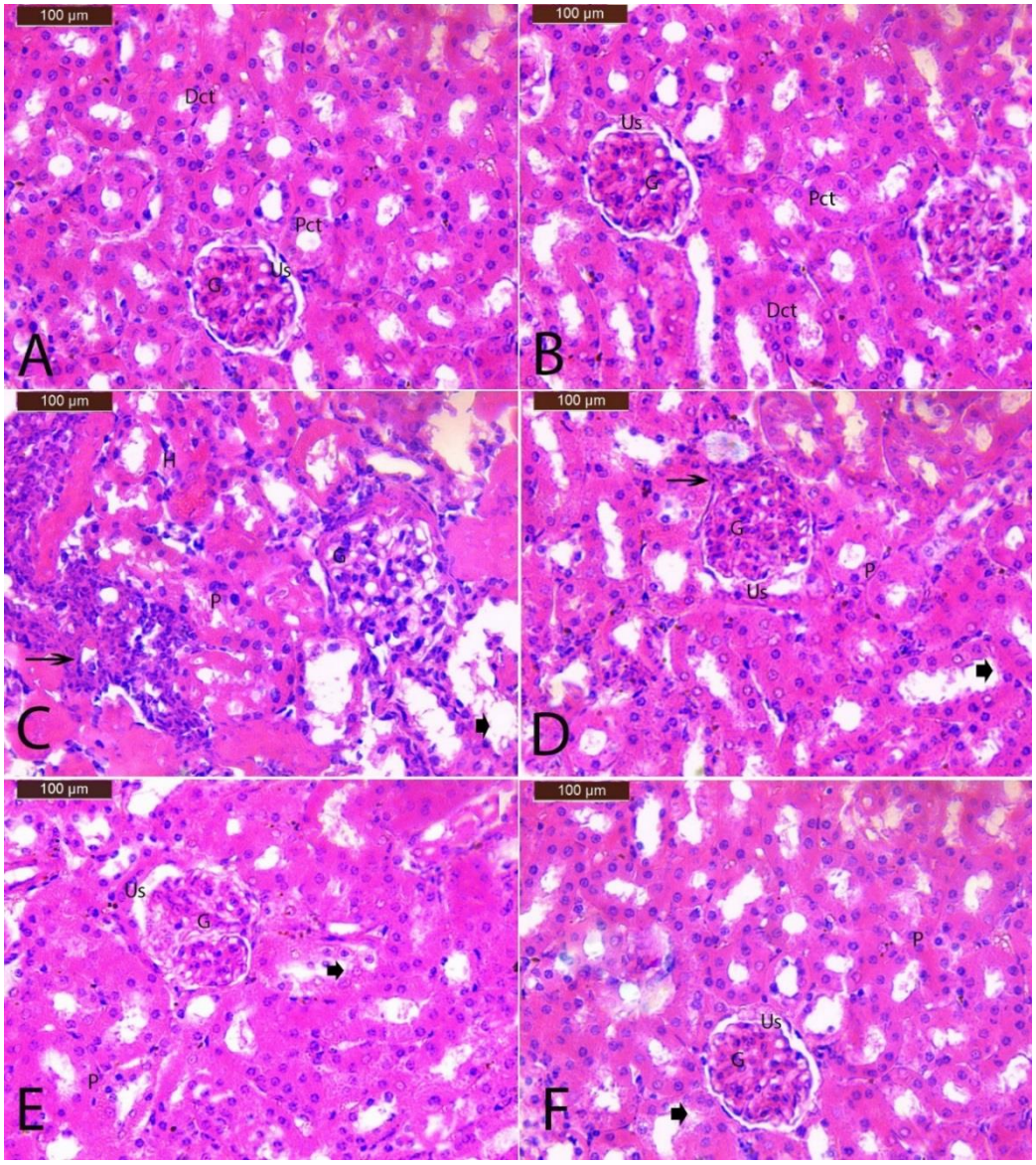

Figure (5): Representative H & E-stained kidney sections showing experimental groups. A: Negative control; B: EGEO-treated group (1.2 mL/kg); C: MMC-treated group; D, E, and F: three co-treated groups with MMC and EGEO at three concentrations 0.8, 1 and 1.2 mL/kg, respectively. Glomerulus (G), Bowman's space (Us), distal convoluted tubules (Dct), proximal convoluted tubules (Pct), inflammatory cells (arrow), necrosis in the tubules (arrowhead), pyknotic nuclei (P), and interstitial hemorrhage (H).

4. Discussion

Anticancer drugs represent the primary therapeutic choice in cancer treatment, and they are employed as a first-line option or as an adjuvant in combined treatments. Chemotherapeutic drugs exert cytotoxicity, genotoxicity, DNA damage and mutagenicity to kill cancer cells. Many anticancer drugs have no selectivity toward cancer cells and have a negative effect on normal cells [18]. Thus, determining the side effects of anticancer drugs is urgently needed. Recently, the search for natural agents that alleviate the side effects of chemotherapeutic agents has been considered a point of interest for many authors [4, 5, 17, 19]. Botanical EOs have possessed wide applications in the field of medicine related to human health. These biological activities of EOS are correlated with their chemical constituents [20].

In this study, GC/MS analysis displayed the presence of 21 constituents, representing 99.54% of the EGEO. Eucalyptol is the primary constituent and represents 74.31%, followed by cymene (6.81%), globulol (4.06%), D-limonene (3.67%), *and* α-pinene (2.12%). The concentration of other components is less than 2%. This result is in line with the outcomes of Elaissi *et al.,* [6]. who stated that eucalyptol is a primary constituent in seven Tunisian species of the genus eucalyptus (*E. leucoxylon, E. cinerea, E. bicostata, E. maidenii, E. lahmannii, E. sideroxylon, and E. astringenso*). Further, eucalyptol is considered an important constituent of EOs of many aromatic plants such as Eucalyptus, Salvia, *Thymus, Rosmarinus, Lavandula, and Cinnamomum* [21, 22].

As expected, MMC induced genotoxic effects in somatic cells, as proved by a significant rise in CAs, MNPCEs, and comet tail consistency in mouse bone marrow. Further, MMC caused several histological distortions in mouse liver and kidney, including necrosis, degeneration, pyknotic nuclei, Kuffer cells, infiltration of inflammatory cells, and hemorrhage. These observations coincide with the previous studies. For example, Krishnaja *et al.,* [23]. reported that MMC induces CAs, sister chromatid exchange, MNPCEs, and comet DNA damage in cultured human blood lymphocytes. Further, MMC induces MN formation in human liver fibroblast cultures [24] and mouse bone marrow [5]. The genotoxic effect of MMC may be related to its ability or its metabolites to bind DNA molecules. Indeed, MMC is an alkylating agent and generates free radicals (ROS) when metabolized. These ROS can attack cellular macromolecules (DNA, RNA, proteins and lipids), leading to DNA damage, cell cycle arrest, and perturbation in DNA repair and apoptosis [25].

As highlighted in our study, EGEO (1.2 mL/kg) exhibited negative genotoxicity in CAs, MNPCEs, and comet DNA damage. Further, EGEO did not produce deleterious histological alterations in liver and kidney sections. Interestingly, EGEO successfully decreased the frequency of CAs, MNPCEs, comet DNA damage, and histopathological lesions induced by MMC in mouse tissues. These data suggest the safety and antigenotoxic activity of EGEO. Our findings are in agreement with previous reports of some chemical constituents of EOs. For example, Mitić-Ćulafić *et al.,* [11]. displayed that eucalyptol reduces the DNA damage induced by the mutagenic agent *t*-butyl hydro-peroxide (*t*-BOOH) in human lymphoma NC–NC and hepatoma HepG2 cell lines. Further, low concentrations (0.05–10 μM) of eucalyptol reduce the comet tail formation in African green monkey kidney cells (Vero cells) induced by 4-nitroquinoline-1-oxide [12]. D-limonene has not been genotoxic and reduced the frequency of MN and DNA damage induced by H2O2 in Chinese hamster lymphocytes V79 cells [13]. D-limonene exhibits sister chromatid exchange and comet tail formation induced by cadmium in human blood lymphocytes. The antigenotoxic activity is associated with the reduction of lipid peroxidation accompanied by elevation in the antioxidant enzymes like catalase and superoxide dismutase [14]. Previous studies showed that globulol did not exhibit any mutagenic effect and showed antimutagenic and antipromutagenic activities against different mutagens in five strains of *Salmonella typhimurium* using the Ames assay [26].

Given the above data, treatment with EGEO at its tested doses (0.8, 1.0 or1.2 mL/kg) were safe and it's pretreated to MMC successfully reversed the genotoxic and histological defects. These observations showed that the protective activity of EGEO is related to its chemical constituents and its antioxidant activity. In this context, Seol and Kim [27] reported that eucalyptol is a promising compound for treating chronic diseases via its potent antioxidant and anti-inflammatory properties. Eucalyptol reduces oxidative stress by regulating signalling pathways and radical scavenging activity [28]. It has been reported that camphor, eucalyptol and thujone modify mutagenesis and DNA repair processes in bacterial system [12]. Similarly, cymene represents a new strategy in developing treatments for many diseases in which oxidative stress plays a main pathophysiological role [29]. Cymene possesses antioxidant properties and reduces the incidence of colon or rectum cancers in the hyperlipidaemia animal model through the reduction of inflammatory cytokines expression and oxidative stress [30]. Generally, multiple pathways may cause antimutagenic properties of monoterpenoids (MTs): MTs inhibit mutagen penetration into cells; MTs stimulate cell antioxidants defence system; MTs scavenges ROS generated by mutagen; MTs inhibit phase I metabolic enzymes involved in the bioactivation of mutagens; MTs activate detoxification phase II metabolic enzymes and MTs interfere with the repair system of DNA [31- 33].

5. **Conclusion**

Our experiments demonstrated that the EGEO has marked anti-genotoxic activity that could be explained by the presence of biologically active constituents such as eucalyptol. It is a dominant constituent of the EGEO and was proven to have anti-genotoxicity, antioxidants, antimutagenic, radical scavenging and DNA repair properties. Other antioxidant compounds with lower concentrations,

such as cymene, globulol, D-limonene, and α-pinene may also synergistically contribute to the Geno-protective activity of EGEO. They all have antioxidant, anticancer, and antimutagenic properties and activate essential antioxidant enzymes. Anti-genotoxic properties of this oil may be considered a critical and promising point in developing new drugs for cancer patients by using this oil.

Conflicts of Interest:

The authors declare no competing interests

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