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Evaluation of the potential protective effect of Commiphora gileadensis on CCL4-induced genotoxicity in mice

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Abstract

Commiphora gileadensis (Arabian balsam) is a wild medicinal plant naturally distributed in Saudi Arabia. To study the effect of habitat variation on the plant chemical compositions, chromatography/mass spectrometry (GC–MS) was applied on the C. gileadensis gathered from two regions (Khulais and Breiman). In addition, the antigenotoxic activity of C. gileadensis were examined by evaluating chromosomal aberrations observed in bone marrow cells, DNA fragmentation in hepatocytes and sperm abnormalities assays in mice. Animal were treated as follow: groups treated with: CCL4 (1.2 mL/kg), high dose of C. gileadensis (400 mg /kg b.w.), low dose of C. gileadensis (200 mg /kg b.w.), CCL4 plus high dose of C. gileadensis, CCL4 plus low dose of C. gileadensis. The results monitored a significant difference between the chemical constitutive of C. gileadensis extracts in the studied regions. In vivo experimentations showed that pretreatment with the tested doses of C. gileadensis succeeded to reduced CCL4 induced injuries. A more efficient protective activity was obtained with implementation of the plant extract at high dose. Thus, the results reflect that the variation in geographical location affect on the chemical compositions of the plant and that C. gileadensis is a promise candidate which could protect against CCL4 induced genotoxicity.

Keywords : Commiphora gileadensis, Gas Liquid chromatography, Genotoxicity, DNA fragmentation, Sperm abnormalities, CCl_4

1. Introduction

Saudi Arabia has a large area with different habitats including mountains, valleys, sandy, and rocky deserts with a characteristic distribution in temperature. [1]. Elsharkawy et al., [2] documented that the geographical location and variation of weather parameters significantly affect plant photosynthesis and metabolism with marked effects on their primary, secondary metabolite giving rise to a great variation in its chemical compositions and biological activity.

Recently, various natural plant products are important alternatives for cancer drugs, antioxidants and antimicrobials due to the potential toxicity of pharmaceuticals and artificial antioxidants [3]. *Burseraceae* family has a hopeful potential for usage as antioxidants and antimicrobials in the pharmaceutical and food industries [4,5]. *Commiphora gileadensis* L. (known as: Arabian balsam or Mecca myrrh) is a medicinal plant that belongs to *Burseraceae* family [6]. It is naturally distributed in Oman, Yemen, and Saudi Arabia. Its secondary metabolites and volatile oils could be applied in medicine and perfume industry [7]. Anciently it was used as a powerful pain and wound treatment [8]. In alternative medicine it was used in treatment of inflammatory, coronary artery and gynecological diseases [9]. Ibrahim et al., [10] illustrated that *C. gileadensis* has a substantial anti-inflammatory effect and possesses protective consequence against diethylnitrosamine-induced liver injury in albino rats. Previous research established that *C. gileadensis* extract exerted antioxidant, antimicrobial, antiviral, antidiabetic, poison antidotes and multiple protective activities. Additionally, anticancer cytotoxic properties were also observed in prostate and liver cancer cell lines [11,12]. Ahmed et al., [13] documented a dose dependent cytotoxicity of C. gileadensis against HepG2 cell line.

Medicinal effects of *C. gileadensis* are principally attributed to the presence of several bioactive constituents including flavonoids, saponins, volatile oils, sterols, triterpenes, β-pinene, β-caryophyllene, and eugenol [14] which may be responsible for its protective and free radical scavenging properties [15].

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C. gileadensis contain terpenoids, as canophyllal, terpinene-4-ol, δ-cadinene and flavonols such as mearnsetin and quercetin which could be responsible for its protective and free radical scavenging activities. [16].

Humans are exposed to industrial toxic substances such as CCL4 via inhalation, oral or dermal routes [17]. CCL4 is a clear, volatile liquid known for its hepatotoxicity and nephrotoxicity through overproduction of reactive oxygen species (ROS), oxidative injury and the inflammatory process [18]. CCL4 has been identified as a group B2, potentially carcinogenic and genotoxic substance to human [19]. So, the present study intended to examine the effects of the geographical variation on the chemical texture of *C. gileadensis* and to provide new insights into its ameliorative activities against CCL4 induced genotoxicity.

2. Materials and Methods

2.1. Sample Collection and Identification of Plant

C. gileadensis samples were gathered from different habitats after leaf emergence (from January to March 2020). The samples were collected from two different sites:

A. Khulais Governorate (22° 3'25.37"N 39°18'1.69"E),

B. Breiman (21°38'34.80"N 39°20'48.81"E),

Samples were identified by Chaudhary [20].

2.2. Preparation of C. gileadensis Extract

Powdered plant shoots of *C. gileadensis* were successively extracted with organic solvents in the increasing polarity order [21]. Each 100g of powder from the plant were soaked and shaken in 300 ml of dichloromethane for 3 days separately. The first filtration process is done by Muslin cloths and Whatman No. 1 filters. Using the Dichloromethane (DCM), the residue was again extracted twice, and the filtrates were combined. The resulting residue was air dried and further extracted with ethyl acetate and followed by ethanol similar to the procedure carried out for the DCM extraction. Under low pressure and temperature, use the rotary evaporator to remove the solvent from the resulting filtrate. Weighted and stored at 4 °C.

2.3. Gas Chromatography–Mass Spectrometry Analysis

Analysis of the *C. gileadensis* extract was performed using the Gas Liquid Chromatography Hewlett Hp 6890 series which included capillary column Hp-5 (phenyl methyl siloxane) with the dimension 30m×0.250umID×0.25um film thickness. Helium was the carrier gas at the rate of 1.0 ml/min. The temperature of the injector was 250 °C and the initial temperature was 45 °C, then increased to 320 °C by the rate of 5.00°C/m. the volume of sample injection was 1 μL on split mode. Using Wiley library to identify the composition.

2.4. In Vivo Antigenotoxic Activity of C. gileadensis

2.4.1. Experimental animals

9-12 weeks old male SWR mice (25-27g each) were obtained from Animal House Colony at King Fahad Medical Research Centre. Animals were reserved in a plastic cage under normal conditions night-day cycle (12/12 h)], food and water were supplied with ad libtium. Animals were kept in artificially illuminated (12 h dark/light cycle) and thermally controlled (25 ± 1°C). All animals received humane care in compliance with the guidelines of the Animal Care and Use Committee (ACUC) at King Fahad Medical Research Centre (KFMRC) with bioethical approval code (31-CEGMR-Bioeth-2021).

2.4.2. Experimental design

Animals were separated into the following groups (five animal per group): Normal control animals, positive control group treated intraperitoneal with CCL4 (1.2 mL/kg dissolved in corn oil), corn oil treated control, two groups were treated orally with high dose (HD) of *C. gileadensis* (400mg /kg b.w.) for 1, 7 days; Two groups were treated orally *with* low dose (LD) of *C. gileadensis* (200mg /kg b.w.) for 1, 7 days; Two groups were treated with CCL4 plus high dose of *C. gileadensis* for 1, 7 days; Two were treated with CCL4 plus low dose of *C. gileadensis* for 1, 7 days.

2.4.3. Chromosomal Abnormalities assay

Examination of chromosomal aberration was directed in the bone marrow cells and spermatocytes of mice intraperitoneally injected with colchicine, two hours before euthanasia [22]. Yosida and Amano [23] technique was applied. One hundred metaphases were examined per animal and the metaphases with aberrations such as gaps, chromosome or chromatid breakage, and fragments were recognized. Chromosome breakage, gaps, and fragments were recorded. The diakinase-metaphase I cells collected from the spermatocytes were examined. XY univalent and autosomal univalent were scored.

2.4.4. Sperm Morphology Assay

The technique of Rasgele [24] were performed to examine anomalies in sperm morphology. Cauda epididymides were crushed in isotonic sodium citrate solution. Sperms were smeared on slides, fixed and stained with Eosin Y [25]. 1000 sperms were examined per animal. The head and tail abnormalities of sperms were detected.

2.4.5. DNA Fragmentation assay

DNA content was calorimetrically detected according to Perandones et al., [26]. Tissues were dissociated in hypotonic lysis buffer (10 mM Tris, 1 mM EDTA, 0.2% Triton X-100, pH 8.0), incubated for half an hour at 48 °C, the pellet (intact chromatin) was separated by centrifugation for 15 min at 12.000 xg. The lysis buffer

was used to resuspend the pellet. 10% trichloroacetic acid was used in the precipitation at 48 OC, centrifuged at 4000 rpm for 10 min, and mixed with 5% trichloroacetic acid then boiled for 15 min and the DNA content was measured using diphenylamine.

2.5. Statistical Analysis

The significance of each result treatments, were measured by t-test. All declarations of significance were recognized on a probability of $p \leq 0.01$

3. Results and Discussion

3.1. Effect of habitat variation on the chemical composition of C. gileadensis

A phytochemical study was performed by GC–MS analysis of *C. gileadensis* extract gathered from different habitats. It is evident that plants gathered from different habitats exhibited various patterns of chemical constituents (Tables 1 and 2). The shared compounds showed significant differences in their concentrations while some compounds were only found in one plant extract but not observed in the plants collected from other habitats Fig (1).

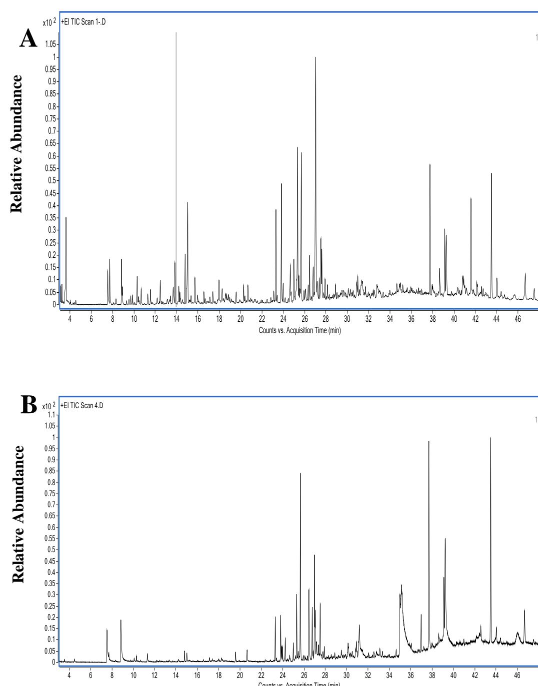

Figure 1. Chromatogram of GC–MS of *C. gileadensis* extract in two regions A: Khulais B: Breiman

Secondary metabolites and antioxidant activity in medicinal plant greatly affected by the environmental factors such as rainfall, temperature and humidity besides the quantity of soil macro and micronutrients [27,28]. Genetic expression of alkaloids [29], terpenoids [30] and phenolic compounds [31] could be modified in stress conditions. In poor nutrient soils, temperature and saline stress, phenolic derivates and antioxidant enzymes, like superoxide dismutase, catalase, peroxidase significantly increased in plant leaves and roots [32].

3.2. Assessment of chromosomal abnormalities in bone marrow

The percentage and number of the chromosomal aberrations observed in control and treated animals were illustrated in Table 3. It was observed that treatment with the low and high doses of *C. gileadensis* for one and seven days is safe, and percentages of abnormal cells were found to be at the normal level. In addition, a highly significant ($p<0.01$) reduction in the proportion of CCL4 induced chromosomal abnormalities were observed 7 days later *C. gileadensis* treatment. The proportion of reduction reached 47.05 and 52.94% after the treatment with LD and HD of *C. gileadensis* respectively (Table 3). Also, Table 3 explains the protective effect of LD and HD of *C. gileadensis* in reducing the diverse types of aberrations.

3.3. DNA fragmentation in hepatocytes

Administration of *C. gileadensis* for 7 days significantly declined the proportion of DNA fragmentation induced by CCL4 in hepatocytes table (4). The high dose was observed to be more effective. The proportion of DNA fragmentation was decreased by 40.68, 52.41 ($P<0.01$) 7 days after treatment with the LD and HD of the tested extracts respectively.

3.4. Spermatocytes and Sperm shape abnormalities

A highly significant ($p<0.01$) reduction in the proportion of CCL4 induced chromosome irregularities in spermatocytes were observed 7 days after *C. gileadensis* treatment. The proportion of reduction reached 45.9 and 54.05% after treatment with LD and HD of *C. gileadensis* respectively (Table 5).

As shown in table (6), CCL4 induced a statistically highly significant elevation in sperm abnormalities in male mice. Amorphous, triangular, without hook heads and coiled tail sperm abnormalities were observed. Simultaneous treatment of mice with low and high doses of *C. gileadensis* reduced the percentage of sperm abnormalities. It reached 8.64, 7.16 % ($p<0.01$) respectively. The percentage of reduction reached 38.28 and 64.57% after treatment of mice with low and high doses of *C. gileadensis* respectively.

In the present study, chromosome abnormalities in bone marrow cells were used as a cytogenetic end point. This technique is a substantial indicator bioassay for the assessment of various environmental pollutants genotoxicity [33].

Table 1. Identification of phytocomponents of C. gileadensis extract in region A: Khulais, using GC– MS analysis

RT	Compound	DB Formula	Area Sum %
7,553	Alpha -Thujene	C10H16	1,1
7,737	ALPHA -PINENE	C10H16	1,25
8,323	Heptenal	C7H12O	0,16
8,839	Beta -Thujene	C10H16	1,05
8,928	Beta-Terpinen	C10H16	0,14
9,713	A-PHELLANDRENE	C10H16O	0,23
9,879	DELTA.3-Carene	C10H16	0,28
10,303	Benzene, 1,2,3,4-tetramethyl	C10H14	0,78
10,425	Sabinene	C10H16	0,14
10,68	1,3,6-Octatriene, 3,7-dimethyl-, (E)	C10H16	0,47
11,308	Gamma -Terpinene	C10H16	0,37
11,554	TRANS-SABINENE HYDRATE	C10H18O	0,52
12,175	Cpd 17 : 12.175		0,25
12,468	Trans Sabinene hydrate	C10H18O	0,85
13,117	1-P-METHYLPHENYL-3-BUTEN-1-OL	C11H14O	0,82
13,846	Bicyclo[3.1.1]hept-3-en-2-ol, 4,6,6-trimethyl-, [1S-(1.alpha.,2.beta.,5.alpha.)]	C10H16O	1,36
14,216	Bicyclo[3.1.0]hexan-2-one, 5-(1-methylethyl)	C9H14O	0,51
14,317	Cpd 26 : 14.317		0,2
14,803	Cpd 28: 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-; C10 H18 O; 14.803	C10H18O	1,69
15,046	PARA-CYMEN-8-OL	C10H14O	3,41
15,35	Cpd 33 : Myrtenol	C10H16O	0,24
15,717	l-Verbenone	C10H14O	0,99
15,973	Carveol 1	C10H16O	0,24
16,569	Benzaldehyde, 4-(1-methylethyl)	C10H12O	0,42
17,123	2-Decenal, (E)	C10H18O	0,18
17,397	3-Hexyne-2,5-diol, 2,5-dimethyl	C8H14O2	0,44
17,978	Benzenemethanol, 4-(1-methylethyl)	C10H14O	0,71
18,263	Phenol, 5-methyl-2-(1-methylethyl)	C10H14O	0,54
18,988	7-Acetyl-5-methyl-2,3-dihydro-1H-pyrrolizine	C10H13NO	0,14
19,576	Alpha -Cubebene	C15H24	0,41
20,289	Alpha -Copaene	C15H24	0,54
20,408	Bicylo[4.1.0]heptane, 7-bicyclo[4.1.0]hept-7-ylidene	C14H20	0,15
20,955	Benzene, 1,2-dimethoxy-4-(2-propenyl)	C11H14O2	0,13
22,843	Beta -Cubebene	C15H24	0,14
23,114	Beta -Selinene	C15H24	0,28
23,315	Sesquisabinene hydrate	C15H26O	3,1
23,423	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1. alpha.,4a. alpha.,8a. alpha.)	C15H24	0,21
23,828	Selina-6-en-4-ol	C15H26O	4,25
23,987	Naphthalene, 1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-, (1S-cis)	C15H22	0,59
25,345	Spathulenol	C15H24O	5,18
25,467	Caryophyllene oxide	C15H24O	0,63
25,561	Beta -copaen-4. alpha.-ol	C15H24O	0,17
25,689	Veridiflorol	C15H26O	5,41
25,947	1,4-Methanoazulen-9-one	C15H24O	0,32
26,331	Apiol	C12H14O4	0,46
26.467	Cubenol	C15H26O	1,46
26,799	T-Muurolol	C15H26O	1,24
27,041	Beta -Eudesmo	C15H26O	11,9
27.37	CALACORENE	C13H16	0,66
27,529	Naphthalene, 1,6-dimethyl-4-(1-methylethyl)	C15H18	1,61
27,612	Longiverbenone	C15H22O	0,9
27,79	Hydroxy-4-isopropylidene-7-methylbicyclo[5.3.1]undec-1-ene; 7-Methyl-4-(1-methylethylidene)bicyclo[5.3.1]undec-1-en-8-ol	C15H24O	0,17
28,14	4-Isopropyl-6-methyl-1,2,3,4-tetrahydronaphthalen-1-one; 1(2H)-Naphthalenone, 3,4-dihydro-6-methyl-4-(1-methylethyl)	C14H18O	0,49
28,743	14-Norcadin-5-en-4-one	C14H22O	0,12
30,314	2-(2-Methoxyphenyl) -1H-pyrrole	C11H11NO	0,51
32,521	(Trans)-2-nonadecene	C19H38	0,33
33,355	Phthalic acid, butyl cyclobutyl ester	C16H20O4	0,19
35,61	1-Dodecanol	C12H26O	0,77
37,749	Gamma-Himachalene	C15H24	4,65
39,1347	Eicosane	C20H42	2,41
39,269	Eicosane	C20H42	3,03
41,587	Alloaromadendrene oxide-(1)	C15H24O	3,56
43,508	Di-(2-ethylhexyl) phthalate	C24H38O4	5,31
44,411	Heneicosane	C21H44	1,59
47,512	Tetracosanoic acid, methyl ester	C25H50O2	0.98

Table 2. Identification of phytocomponents of C. gileadensis extract in region B: Breiman, using GC– MS analysis.

RT	Compound	DB Formula	Area Sum %
7,539	Alpha -Thujene	C10H16	2,7
7,712	BETA-OCIMENE	C10H16	0,24
8,845	Beta -Thujene	C10H16	3,97
10,085	Alpha -Terpinene	C10H16	0,17
10,309	P-Methylcumyl Alcohol	C10H14O	0,25
11,325	L-Phellandrene;	C10H16	0,47
14,811	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)	C10H18O	
15,029	PARA-CYMEN-8-OL	C10H14O	0.52
19,588	Alpha -Cubebene	C15H24	0,62
20,668	Beta -Cubebene	C15H24	0,78
23,817	Selina-6-en-4-ol	C15H26O	2.48
25,221	2,2,4-Trimethyl-4-pentenenitrile	C8H13N	1.06
25,318	Spathulenol	C15H24O	4.67
25,663	Veridiflorol	C15H26O	9.63
26,465	Cubenol	C15H26O	3.95
26,77	T-Muurolol	C15H26O	2.82
26,991	Beta -Eudesmo	C15H26O	3.35
27,06	POGOSTOL	C16H28O	1.11
27,358	CALACORENE	C13H16	0.55
27,517	Naphthalene, 1,6-dimethyl-4-(1-methylethyl)	C15H18	3.14
28,895	2.BETA. -T-BUTYL-1,2,3,4,4A. BETA.,5,6,7,8,8A. BETA. -DECAHYDRONAPHTHALEN-2. ALPHA. -OL	C14H26O	0.39
31,178	Tetradecanoic acid,	C17H36O2Si	2.69
33,354	1,2-Benzenedicarboxylic acid, dibutyl ester	C16H22O4	0,55
34,991	hexadecanoic acid tert-butyl-l ester;		0,5
35,13	hexadecanoic acid tert-butyl-l ester	C22H46O2	0,19
37,707	Gamma -Himachalene	C15H24	21.43
39,338	2- and 4-(Trimethoxyacetoxy)-2,5,5-trimethyl-3-hexanone	C14H26O3	3,31
39,364	1-Isopropoxy-2-methyl-2-phenyl-3-(phenylimino)indoline	C24H24N2O	0,26
42,455	Heptanedioic acid, 4,4-dicyano-2,6-bis(methoxyimino)-, diethyl ester	C15H20N4O6	0.59
42,575	pentadecane	C15H32	0.72
43,496	Di-(2-ethylhexyl) phthalate	C24H38O4	15.18
44,411	(2R,3R,4R,5S,6R,8S,10S) -5-acetoxy-10-benzyloxy-8-benzyloxymethyl-2-ethyl-3-methyl-1,7-dioxaspiro [5.5] undecan-4-ol ; 1,7-Dioxaspiro [5.5] undecane-4,5-diol, 2-ethyl-3-methyl-10-(phenylmethoxy) -8-[(phenylmethoxy)methyl] -, 5-acetate, [2R-[2.alpha.,3.beta.,4.bet	C29H38O7	0.94
46,667	Nonadecane	C19H40	3.21

Table 3. Different forms of chromosomal aberrations in bone marrow cells after treatment with the two doses of *C. gileadensis* alone or in combination with CCL4.

Treatment and doses (mg/kg b.wt.)	Treatment day(s)	No. of metaphases with				Chromosomal aberrations		Inhibition %
		Gap	frag. and /or break	Deletion	Total	Excluding gaps Mean	including gaps	
CCL4	1	26	36	6	68	8.4 ± 0.7^{a}	13.6 ± 0.9^{a}	
Control		2	4	0	6	0.8 ± 0.4	1.2 ± 0.4	
Corn oil		4	6	2	12	1.6 ±0.4	2.4 ± 0.4	
LD *C. gileadensis*		6	6	2	14	1.6 ± 0.4	2.8 ± 0.4	
HD *C. gileadensis*		6	6	2	14	1.6 ± 0.4	2.8 ± 0.48	
LD+CCL4		10	36	6	52	8.4 ± 0.4^{a}	10.4 ± 0.4^{b}	23.5
HD+CCL4		14	32	4	50	7.2 ± 0.48^{a}	10.0 ± 0.1^{b}	26.4
CCL4	1	26	36	6	68	8.4 ± 0.7^{a}	13.6 ± 0.9^{a}	
Control	7	2	4	0	6	0.8 ± 0.48	1.2 ±0.4	
Corn oil		4	6	2	12	1.6 ± 0.4	2.4 ± 0.4	
LD *C. gileadensis*		6	8	0	14	1.6 ± 0.7	2.8 ± 0.4	
HD *C. gileadensis*		4	8	0	12	1.6 ± 0.43	2.4 ± 0.4	
LD+CCL4		14	20	2	36	4.4 ± 0.4^{ab}	7.2 ± 0.48^{ab}	47.05
HD+CCL4		10	22	0	32	4.4 ± 0.4^{ab}	6.4 ± 0.4^{ab}	52.94

a: significant at 0.01 level (t-test) compared to control (non-treated).
b: significant at 0.01 level (t-test) compared to treatment (CCL4)

Table 4. DNA fragmentation in hepatocytes after treatment with the two doses of *C. gileadensis* alone or in combination with CCL4.

Treatment and doses (mg/kg b.wt.)	Treatment Day(s)	DNA fragmentation Mean% ±S.E.	DNA fragmentation inhibition %
CCL4	1	8.7±0.4 [a]	
Control		3. 0±0.27	
Corn oil		3.2±0.6	
LD *C. gileadensis*		2.9±0.31	
HD *C. gileadensis*		3.23±0.16	
LD+CCL4		8.2±0.62	5.74
HD+CCL4		8.32±0.32	4.36
CCL4	1	8.7±0.4 [a]	
Control	7	3. 0±0.27	
Corn oil		3.0±0.35	
LD *C. gileadensis*		2.89±0.29	
HD *C. gileadensis*		3.07±0.22	
LD+CCL4		5.16±0.41 [b]	40.68
HD+CCL4		4.14±0.2 [b]	52.41

a: significant at 0.01 level (t-test) compared to control (non- treated). b: significant at 0.01 level (t-test) compared to treatment (CCL4)

Table 5. Different types of chromosomal aberrations in mouse spermatocytes after treatment with the two doses of *C. gileadensis* alone or in combination with CCL4.

Treatment and doses (mg/kg b.wt.)	Treatment day(s)	No. of metaphases with			Total aberrations		Inhibition %
		XY Univalent	Autosomal univalent	XY+ Autosomal Univalent	No.	Mean%±. S.E.	
CCL4	1	19	9	7	35	7.0 ±0.37[a]	
Control		11	4	0	15	3.0 ± 0.12	
Corn oil		9	5	0	14	2.8 ± 0.2	
LD *C. gileadensis*		10	4	0	14	2.8 ± 0.2	
HD *C. gileadensis*		9	4	1	14	2.8 ± 0.18	
LD+CCL4		20	10	4	34	6.8 ±0.36	2.8
HD+CCL4		21	9	5	35	7.0 ± 0.31	
CCL4	7	21	9	7	37	7.4 ±0.41[a]	
Control		10	5	0	15	3.0 ±0.41	
Corn oil		11	4	1	16	3.2 ± 0.21	
LD *C. gileadensis*		8	4	1	13	2.6 ± 0.2	
HD *C. gileadensis*		9	3	0	12	2.4 ± 0.19	
LD+CCL4		11	6	3	20	4.0 ±0.22[ab]	45.9
HD+CCL4		10	5	2	17	3.4 ± 0.23^{ab}	54.05

a: significant at 0.01 level (t test) compared to control (non- treated).
b: significant at 0.01 level (t test) compared to treatment (CCL4)

Table 6. Number and proportion of different types of sperm shape anomalies after treatment with the two doses of *C. gileadensis* alone or in combination with CCL4.

Treatment and doses (mg/kg b.wt.)	Examined sperm No.	No. of sperms with				Abnormal sperm No.	Abnormal sperms Mean % ± S.E.	Inhibition %
		Head abnormalities			Tail abnormalities			
		Amorphous	Triangle	Without hook	Coiled			
CCL4	5000	111	136	90	13	350	7.0 ± 0.83^{a}	
Control	5000	28	36	28	5	97	1.94 ± 0.81	
Corn oil	5000	28	36	28	5	97	1.94 ± 0.81	
LD *C. gileadensis*	5000	27	34	26	4	91	1.82 ± 0.58	
HD *C. gileadensis*	5000	29	35	29	1	94	1.88 ± 0.58	
LD+CCL4	5000	65	95	50	6	216	4.32 ± 0.71^{ab}	38.28
HD+CCL4	5000	30	57	35	2	124	2.48 ± 0.3^{ab}	64.57

a: significant at 0.01 level (t test) compared to control (non- treated).
b: significant at 0.01 level (t test) compar

A highly significant proportion of chromosomal anomalies in mouse bone marrow cells were observed in CCL4-treated groups. Chromosomal fragments, breaks and deletions were observed. CCL4 also significantly increased the mean percentages of sperm shape abnormalities. In addition, the proportion of DNA damage in liver cells increased to 8.7 % (P > 0.01) comparing to 3.0% for the control group. Likewise, the current observations, CCl4 were documented to induce chromosomal aberration both in vitro [34] and in vivo in somatic and germ cells [19]. CCl4 binds to hepatic cytochrome P450 to form secondary metabolites and free radicals such as nitric oxide and peroxynitrite, which initiate lipid peroxidation, DNA damage, base substitution, deletion, and strand fragmentation [35-37]. Depletion of antioxidant enzymes, and enhancement of lipid peroxides, nitrite content, and hydrogen peroxide contribute to the CCL4 induced liver and testis injuries [38].

The more recent scientific studies greatly focused on the potential applications of medical, pharmaceutical and food industries of secondary plant metabolites [39]. Plant extracts as natural sources of antioxidants, are powerful substances which scavenge free radicals and inhibit the cellular oxidative damage [40]. The present results demonstrated a significant reduction in the aberrant bone marrow cells, DNA damage in hepatocytes and sperm abnormalities induced by CCL4 after administration of *C. gileadensis* for 7 days. A more efficient protective activity was obtained with implementation of the plant extract at high dose. In accordance, Rezaei et al., [41] revealed that treatment with *Commiphora* extract increased sperm motility and viability in diabetic rats. The antioxidant and scavenging activities of *C. gileadensis* could be attributed to its high content of tocopherols, phytosterols, and phenolic compounds [13]. *C. gileadensis* could improve the hepatic functions by prevention of reactive metabolite formation through direct inhibition of the hepatic cytochrome P450. In addition, repression of lipid peroxidation stabilizes the hepatocyte membrane and enhances protein synthesis [42].

4. Conclusion

The more recent scientific studies greatly focused on the potential applications of medical, pharmaceutical and food industries of secondary plant metabolites. *C. gileadensis* were gathered from two regions (Khulais and Breiman). The results reflect that the variation in geographical location affect the *C. gileadensis* chemical compositions in the two studied regions. The present study also showed that administration of *C. gileadensis* significantly declined the proportion of chromosomal abnormalities in bone marrow cells, DNA fragmentation in hepatocytes and sperm shape anomalies induced by CCL4. *C. gileadensis* could likely be a powerful remedy to optimize the deteriorative properties of different chemical-induced oxidative damage and cytogenetic abnormalities. The experimental work presented here provides one of the first investigations on the antigenotoxic properties of *C. gileadensis*. Nevertheless, continued efforts are needed to identify the mechanisms engaged in these antigenotoxic activities.

Author Contributions:

This work was carried out through the collaboration of all authors. Author Asmaa S. Salman, wrote the protocol, carried out the experimental work of the genetic part and managed the literature searches, wrote the final draft of the manuscript. Author Emad A. Alsherif1 prepared plant materials, carried out the GC–MS analysis and managed the literature searches. Author Seham M. Althobiti performed the statistical analysis, carried out the experimental work of plant materials, GC–MS analysis and genetic part of the work. All authors read and approved the final manuscript.

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