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Cymbopogon citratus essential oil alleviates the genotoxicity and oxidative stress of carbon tetrachloride in mice

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Abstract

Nowadays, there is an increasing trend to use aromatherapy for treatment of various diseases. *Cymbopogon citratus* (Lemongrass) is one of many plants that have been reported to employ successfully in aromatherapy. Herein we decided to explore the protective role of *Cymbopogon citratus* essential oil (CCEO) against hepatic/renal damage and genotoxicity induced by carbon tetrachloride (CCl_4) and the relation of this bioactivity with its chemical constituents. Six main groups of mice (five/each) were examined: I- represents negative control group, II and III- mice received oral treatment with CCl_4 (1mL/kg, positive control) and CCEO (0.3 mL/kg, control plant) respectively for five consecutive days and IV-VI- represent groups of mice treated with CCEO at the three concentrations 0.1, 0.2, 0.3 mL/kg plus CCl_4 (five consecutive days treatment). Remarkable adverse effects of CCl_4 in all the tested parameters were recorded. These effects were distinguished as an increment in the level of all liver marker enzymes (ALT, AST, ALP, γ-GT), blood urea, and creatinine. Also, the oxidative stress biomarkers: malondialdehyde (MDA) and glutathione-transferase GST were affected after CCl_4 treatment. Regarding the genotoxic effect of CCl_4, the percentage of chromosomal aberrations in bone marrow and spermatocyte cells was elevated ($p< 0.05$) compared with the negative control. Notable antioxidant, hepatic/renal protection and anti-mutagenic potency of CCEO against CCl_4 were demonstrated with a dose-related relationship. GC/MS analysis demonstrated the presence of 12 phytochemical constituents which in combination play a critical role in its antioxidant/antigenotoxic efficacy. The major components exist were E. Citral (35.13%) and Geraniol (32.83%).

Keywords: *Cymbopogon citratus,* hepatic /renal protection, antimutagenic, carbon tetrachloride, mice

Introduction

Carbon tetrachloride (CCl_4) is utilized in many industrial applications. It is used as a precursor to refrigerants and as a cleaning agent. As a solvent, it is well suited to dissolving other non-polar compounds, fats, oils, rubber waxes, lacquers, and varnishes. It is also used as grain fumigant, as an extracting solvent for flowers and seeds and as a component in fire extinguishers. CCl_4 also has an anthelmintic property and act as anesthetic agent (ATSDR, 1994).

CCl_4 is present in the environment because it does not break down easily and has built up over time from human activities. The poisonous nature of CCl_4 is well established; it's hepatotoxic and damaging agent to the kidney, central nervous system and ultimately carcinogenic (IRIS, 2010). CCl_4 exerts its toxicity through bio-activation of the trichloromethyl radical that can covalently bind to macromolecules or enhanced lipid peroxidation that mediated cellular damage. CCl_4 can also induce the activation of macrophages, and the release of many pro-inflammatory cytokines, including tumor necrosis factor-a (TNF-a), irreducible nitric oxide synthase (iNOS), nuclear factor-kappa B (NF-kB) and interleukin-1β (IL-1β). The above oxidative stress and inflammatory factors participate in the process of acute hepatic injury and other toxicities associated with CCl_4 uses (Chen et al., 2017).

Nowadays, natural products have considerable attention related to their important role in the prevention and/or inhibition of free radical generation and oxidative stress resulting from extensive exposure to exogenous agents (MacHraoui et al., 2018). Furthermore, many epidemiological studies demonstrated the association of dietary intakes of vegetables, fruit, cereal, and teas with a lower risk of several human diseases and cancers. Concerning liver diseases, as an example, herbal drugs

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were reported to play a significant and remarkable role in the healing process and management of many liver disorders through acceleration and regeneration of liver cells (Ahmad et al., 2006). Also in the immune system, renal and cancer diseases, the protective role of many phytochemicals was recorded in different scientific research (Jha, 2010; Khodadadi, 2016).

Lemongrass is one of the herbal plants that used in many countries as traditional folk medicine. Its effectiveness against oxidative damage and cancer have been documented (Ghosh, 2013). Also, lemongrass essential oil was reported to have various *in vitro* and *in vivo* pharmacological activities, including anxiolytic and anticonvulsant activities (Satthanakul et al., 2015; Ahmad & Viljoen, 2015). The antioxidant activities of lemongrass extracts were reported in different systems (Jamuna et al., 2017).

In the current work, a comprehensive study was undertaken to demonstrate the protective effect of lemongrass essential oil against hepatic/renal damage and genotoxicity induced by CCl_4. The correlation of this bioactivity with its chemical constituents was also discussed.

Material and Methods

Chemical and kits

All chemicals and reagents used in this study were obtained from the commercial sources as following: Carbon tetrachloride (CCl4) was supplied from Alpha Chemika (Mumbai, India). Kits for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), gamma-glutamyltransferase (γ-GT), Urea and creatinine were purchasd from (Spectrum®, Hannover, Germany). Malondialdehyde (MDA) ELISA Kit was purchased from (OxiSelect™ HNE Adduct Competitive, Cell Bio labs, Inc. San Diego, CA, and USA). Total glutathione *S-transferase* (GST) kit was purchased from (Cayman chemical *company, Ann Arbor, MI, USA*. *Colchicine. BDH Laboratory Supplies (England) Product No.: 27805 FM, Potassium chloride (Purified Kcl)Product NO.: 39594.S.d.FiNE-CHEM LTd, Tri-Sodium Citrate 2-Hydrate. C6H5Na3O7.2H2O - PA-ACS (Panreac Quimica SA), Giemsa´s Stain MS.Product No.: 44034 S.d. S.d.FiNE-CHEM LTd.*

Extraction of essential oil from Cymbopogon citratus:

Plant samples of *Cymbopogon citratus* were collected from the Experimental Agricultural Station, Fac. of Agriculture, Cairo University during the season of 2017-2018. Botanical identification was carried by by Prof. Dr. Ahmed Shalaby, Prof. of Medicinal and Aromatic Plants, National Research Centre, Dokki, Giza and the percentage of the essential oil was calculated.

Chemical investigation of Cymbopogon citratus essential oil

Determination of volatile oil content:

Plant samples were used for the determination of volatile oil content. The volatile oil of the fresh sample was extracted by the water distillation method (for 3 hrs.) in a Clevenger's apparatus (Guenther, 1953). The sample was done in triplicate and the mean values of the oil content (%) were recorded.

Identification of the chemical composition of Cymbopogon citratus essential oil

Gas chromatography–mass spectrometry analysis (GC-MS)

The GC-MS system (Agilent Technologies) was equipped with gas chromatograph (7890B) and 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 HP-5MS column (30 m x 0.25 mm internal diameter and 0.25 µm film thickness). Analysis was carried out using helium as the carrier gas at a flow rate of 1.0 ml/min at a split ratio of 1:30, injection volume of 1 µl and the following temperature program: 40 °C for 1 min; rising at 4 °C /min to 150 °C and held for 6 min; 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 (EI) at 70 eV and using a spectral range of m/z 50-550. The identification of different constituents was determined by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST Mass Spectral Library data.

Experimental animals

Male mature albino mice of Swiss strain weighing 25-30 g were obtained from the animal house, National Research Centre, Egypt. Animals were housed in an ambient temperature of (25 ± 3) °C on light/dark cycle of 12/12 hours. All mice were kept in clean polypropylene cages and administered food and water *ad libitum*.

Ethical consideration

This prospective study was reviewed and approved by the animal ethics committee of the National Research Centre, Cairo, Egypt (approval number:1.6.2.1.0) and carried out according to the

National Institute of Health Guide (NIH) for the care and use of laboratory animal's guidelines.

Experimental design and doses

Thirty mice were divided into six groups (five animals/ each) and treated for 5 consecutive days as follows: Group I, negative control.; Group II, mice treated orally with CCl_4 (1mL/kg b.w);Group III; mice treated orally with CCEO (0.3 mL/kg b.w) Groups IV-VI, mice treated orally with CCEO (0.1, 0.2 and 0.3 mL/kg b.w) + CCl4 (1mL/kg b.w). Selected doses for CCEO and CCL4 were determined according to Gebremickael (2017) and Diab et al. (2018) respectively. For the tested groups (IV-VI) mice were received CCEO one hour before CCl4.

Experimental procedures:

Biochemical assays:

A blood sample from each mouse was collected by retro-orbital puncture from orbital plexus using blood capillary tubes. Samples were collected in clean dry test tubes, allowed to clot and then centrifuged at 3000 rpm for 15 minutes. The separated serum was collected in clean stopper plastic vials and kept at -8°C until the analysis of serum parameters.

Genotoxicity assays

Chromosomal aberration assay in mouse bone marrow and spermatocytes

Bone marrow and spermatocyte chromosomes were prepared according to the technique described by Fahmy et al. (2017). One hundred well- spread metaphases were analyzed per mouse describing different kinds of abnormalities. In bone marrow and spermatocytes scoring was performed under 2500× magnification with a light microscope.

Statistical analysis

Data were computerized and analyzed using the Statistical Package of Social Science (SPSS Inc., version 20, Armonk, New York: IBM Corp). One way analysis of variance (ANOVA) followed by Duncan's multiple comparison test was used to determine the difference among the means. The level of statistical significance was set at $P < 0.05$.

Evaluation of the effect of CCEO to inhibit DNA damage induced by CCl_4 was carried out according to Al-Ashaal et al. (2017) equation as follows:

Inhibitory index (II) = [1- ((CCEO plus CCl_4) – control) / (CCl_4- control)] ×100.

Results

Analysis of chemical constituents by GC/MS

According to GC/MS investigation, total of twelve phytochemical constituents of lemongrass essential oil was identified. The major components exist, E. Citral (35.13%); Geraniol (32.83%); β-Myrcene (8.69%); Nerol (5.60%); Geranyl acetate (3.48); isoneral (2.63%); linalool (2.04%) and others (Table1).

Table 1. GC/MS analysis of Cymbopogon citrates essential oil.

Peak	Compound	Rt	Conc. %
1	β-Myrcene	10.14	8.69
2	Myroxide	13.78	0.40
3	Linalool	14.02	2.04
4	Isoneral	16.34	2.63
5	Citronellol	18.77	0.94
6	Geraniol	19.18	32.83
7	Nerol	19.64	5.60
8	E-Citral	20.25	35.13
9	Geranyl acetate	23.78	3.48
10	cis-a-Bergamotene	25.39	0.70
11	Caryophyllene oxide	29.94	0.51
12	Cadinene	31.25	0.95
	Unknown		6.11
	Oxygenated compounds		83.56
	Non oxygenated compounds		16.44

Rt: Relative retention time

Biochemical Studies

The results in Table (2) showed that mice treated with CCEO alone at the highest tested dose (0.3 mL/kg b.w.) had no statistical changes in the frequency of liver enzymes (AST, ALT, LDH, ALP and **γ**-GT) as compared to control negative. However, treatment with CCl_4 showed a significant increase in the level of all liver enzymes. This elevation decrease with different doses of CCEO when administrated in combination with CCl_4. Dose-dependent protection was recorded. Also an increase in MDA and a remarkable decline in GST levels were observed after CCl_4 treatment (Table 3). The oxidative damage was

reversed by lowering MDA level and heightening GST levels when CCEO was given in combination with CCl_4. It is worth mentioning that CCEO alone at the highest tested dose had a normal effect on both MDA and GST levels compared to control negative. Treatment with CCl4 showed that the levels of urea and creatinine were significantly increased (Table 3). The combined treatment with CCE succeeded to induce a significant improvement in urea and creatinine level to the normal level at the highest tested dose of CCEO.

Table 2. Effect of *Cymbopogon citrates* essential oil (CCEO) and carbon tetrachloride on liver function markers.

	Liver function markers				
Experimental groups	ALT (u/l)	AST (u/l)	ALP (u/l)	LDH (u/l	γ-GT (u/l)
Group I	5.9 ± 2.8^{a}	7.2 ± 1.5^{a}	81.5 ± 6.22^{a}	9.5 ± 3.25^{a}	0.66 ± 0.07^{a}
Group II	5.0 ± 2.37^{a}	7.51 ± 1.62^{a}	78.8 ± 2.55^{a}	10.99 ± 3.11^{a}	0.79 ± 0.64^{a}
Group III	77.0 ± 6.44^{ef}	119.0 ± 7.64^{ef}	344.00 ± 10.78^{ef}	511.38 ± 9.71^{fg}	4.0 ± 1.29^{de}
Group IV	29.0 ± 2.74^{cd}	55.7 ± 4.92^{cd}	167.25 ± 11.87^{cd}	75.38 ± 5.11^{cd}	7.18 ± 0.58^{bc}
Group V	22.0 ± 3.37^{cd}	36.27 ± 5.99^{bc}	137.3 ± 12.77bc	59.43 ± 6.17^{cd}	5.41 ± 0.75^{bc}
Group VI	19.5 ± 4.34^{bc}	22.14 ± 4.48^{bc}	98.2 ± 17.48^{ab}	24.15 ± 4.35^{b}	4.48 ± 0.78^{b}

The data were presented as mean ± SE (n=5).The values having different superscript letters in each column are significantly different from one another as calculated by ANOVA (P<0.05). Group I, negative control; Group II, CCEO (0.3 mL/kg b.w); Group III, CCl4 (1 mL/kg b.w); Groups IV, CCEO (0.1 mL/kg) + CC14; Group V, CCEO (0.2 mL/kg) + CC14 and Group VI, CCEO (0.3 mL/kg) + CC14

Table 3. Effect of Cymbopogon citrates essential oil (CCEO) and carbon tetrachloride on kidney function and oxidative stress markers.

	Kidney function markers		Oxidative stress	
Experimental groups	S. Urea(mg/dl)	S. Creatinine(mg/dl)	GST (µg/ml)	MDA (pmol/ml)
Group I	29.0 ± 5.2^{a}	0.64 ± 0.03^{a}	1.98 ± 0.23^{a}	1.81 ± 0.49
Group II	26.27 ± 4.31^{a}	0.66 ± 0.05^{a}	2.59 ± 0.64^{a}	1.99 ± 0.47
Group III	69.88 ± 2.64^{de}	2.54 ± 0.27^{bc}	0.8 ± 0.15^{cd}	15.0 ± 1.7^{de}
Group VI	39.08 ± 3.8^{ab}	1.2 ± 0.08^{b}	1.5 ± 0.20^{ab}	5.78 ± 0.84^{bc}
Group V	38.22 ± 6.0^{ab}	0.92 ± 0.07^{ab}	1.7 ± 0.56^{a}	4.5 ± 0.62^{bc}
Group IV	31.0 ± 22^{a}	0.77 ± 0.09^{a}	1.9 ± 0.88^{a}	2.12 ± 0.38^{a}

The data were presented as mean ± SE (n=5).The values having different superscript letters in each column are significantly different from one another as calculated by ANOVA (P<0.05). Group I, negative control; Group II, CCEO (0.3 mL/kg b.w); Group III, CCl4 (1 mL/kg b.w), Groups IV, CCEO (0.1 mL/kg) + CC14; Group V, CCEO (0.2 mL/kg) + CC14 and Group VI, CCEO (0.3 mL/kg) + CC14

Cytogenetic studies

Chromosomal aberrations analysis in the bone marrow and spermatocyte cells:

Figure (1) represents the mean percentage of chromosomal abnormalities induced by CCl_4 and CCEO in bone marrow and mouse spermatocytes. The percentage of aberrations significantly did not change after treatment with the highest dose of CCEO in mouse somatic and germ cells compared with the negative control. While the levels of chromosomal abnormalities elevated ($P< 0.05$) in CCl_4 treated group compared with the negative control. That results also showed that the oral administration of CCEO at the three tested concentrations (0.1, 0.2 and 0.3 mL/kg.) with CCl_4 reduced chromosome damage ($P<0.05$) induced by CCl_4 in a dose-dependent manner. The reduction of aberrations reached 31, 51, and 62 % in bone marrow cells and 35, 48 and 55 % in spermatocyte cells after treatment with three tested doses respectively. Table (4) shows the number and percentage of different types of chromosome abnormalities induced in somatic and germ cells.

Discussion

The treatment of animals with CCl_4 at the tested concentration induced hepatic and renal dysfunction. These effects were evidenced by increases in the level of liver enzymes ALT, AST, ALP, and γ-GT, together with blood urea, creatinine. The results also demonstrated the oxidative stress after CCl_4 treatment evidenced by significant increases in MDA and a decrease in GSH. These results are supported by the previous work of other authors who demonstrated the liver dysfunction after CCl_4 treatment (Qing et al., 2017). The elevated levels of serum parameters ALT, AST, ALP and γ-GT are a direct reflection of the loss of liver function through cellular leakage and alterations in the hepatic structural integrity (Adewale et al., 2014). These liver enzymes are located in the cell cytoplasm and are emptied into the circulation once the cellular membrane is damaged (Lin & Huang, 2000). The increase in the serum level of ALT enzyme, in particular, is an indicative tool of liver damage. Creatinine level was reported to be an ideal endogenous substance for measuring glomerular filtration rate (Traynor et al., 2006). The Kidney was reported to be more vulnerable

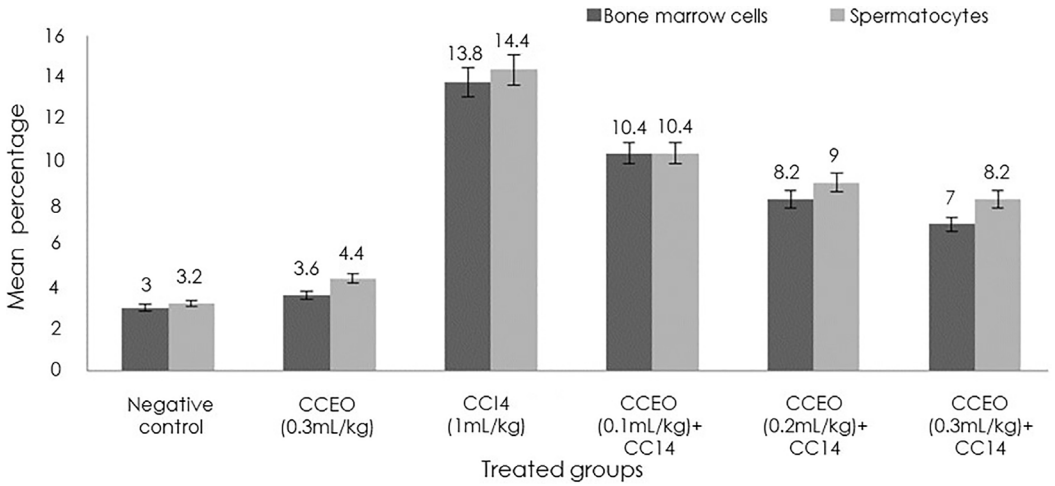

Figure 1. Mean percentage of metaphases with chromosomal abnormalities induced in mouse bone marrow (somatic cells) and spermatocytes (germ cells) after treatment with CCl_4 and CCEO.

Table 4. The effect of CCEO on CCl_4-induced chromosomal aberrations in mouse bone marrow and spermatocyte cells.

Experimental groups	Bone marrow					Inhibitory index	Spermatocytes					Inhibitory index
	Abnormal Metaphases		No. and (%) of different types of abnormal metaphases				Abnormal Metaphases		No. and (%) of different types of abnormal metaphases			
	No.	Mean % ± SE	Gap	Fragment and/or Break	Deletion		No.	Mean % ± SE	XY-uni.	Auto. uni	XY uni+ Auto.uni	
Group I	15	3.00 ± 0.58^{a}	7 (1.4)	7 (1.4)	1 (0.2)	-	16	3.20 ± 0.64^{a}	13 (2.6)	3 (0.6)		-
Group II	18	3.60 ± 0.50^{a}	8 (1.6)	9 (1.8)	1 (0.2)	-	22	4.40 ± 0.50^{a}	14 (2.8)	8 (1.6)	-	-
Group III	69	13.80 ± 0.67^{de}	15 (3.0)	47 (9.4)	7 (1.4)	-	72	14.40 ± 0.5^{de}	38 (7.6)	29 (5.8)	5(1.0)	-
Group IV	52	10.40 ± 0.62^{cd}	7 (5.4)	42 (8.4)	3 (0.6)	31	52	10.40 ± 0.40^{cd}	31 (6.2)	18 (3.6)	3(0.6)	35
Group V	41	8.20 ± 0.50^{bc}	9 (1.8)	28 (5.6)	4 (0.8)	51	45	9.00 ± 0.58^{bc}	34 (6.8)	10 (2.0)	1(0.2)	48
Group VI	35	7.00 ± 0.45^{bc}	6 (1.2)	27 (5.4)	2 (0.4)	62	41	8.20 ± 0.72^{bc}	27 (5.4)	14 (2.8)	-	55

Number of examined metaphases = 500 (100 metaphase/animal, 5 animals/group). XY-uni: XY- univalent; Auto. uni.: Autosomal univalent. The values having different superscript letters in each column are significantly different from one another as calculated by ANOVA.

to oxidative stress than liver (Suzuki et al., 2015). In this respect, Rincón et al. (1999) reported that the effect of CCl_4 on kidney structure and function depends on the functional state of the liver. Ozturk et al. (2003) suggested that liver is not only the target organ affected by CCl_4 but it also affects several organs of the body such as kidney, testes, lung, heart, and brain. Inflammation and oxidative stress are the main pathways of CCl_4-induced toxicity (Alshammari et al., 2017). CCl_4 is metabolized in the liver by the cytochrome P450 enzyme leading to a highly reactive trichloromethyl free radical (•CCl3) and/or trichloromethylperoxyl radical (•OOCCl3). Such metabolites induce oxidative stress and trigger the production of lipid peroxidation which sequentially attacks hepatic tissue. Moreover, CCl_4 could trigger the production of inflammatory chemokines and cytokines, stimulating the induction of inflammatory cells (Chen et al., 2017).

In the present study, the protective effect of CCEO is investigated. The role of any hepatoprotective agent is indeed dependent on its capability of either reducing the toxic effects or in maintaining the normal hepatic physiological mechanism which has been imbalanced by hepatotoxin. CCEO depleted the elevated liver marker enzymes in CCl_4-treated mice to nearly normal values. Moreover, CCEO significantly decreased the elevated levels of blood urea and creatinine, which indicates the protection of kidney tissue against oxidative damage and maintenance of renal function. The results are supported by Koh et al. (2012) who demonstrated that *Cymbopogon citratus* extract alleviated hepatic damage induced by CCl_4 in rats dose-dependently ($p< 0.05$) through decreasing in the CCl_4-elevated levels of serum biochemical parameters, malondialdehyde (MDA) level, and increase in GSH and antioxidant enzymes. The authors suggested that *Cymbopogon citratus* has antioxidant and free radical scavenging property. Also, Rahim et al. (2014) demonstrated that *C. citratus* aqueous extract could effectively alleviate H_2O_2-induced oxidative stress and prevent liver injury in male rats and at the same line Uchida et al. (2017) found hepatoprotective activity against paracetamol liver toxicity. Also, Luís et al. (2017) reported that *Cymbopogon citratus* essential oil has a powerful capacity to scavenge the DPPH free radicals.

Furthermore, Gbenou et al. (2013) demonstrated that the essential oil of *Cymbopogon citrates* has an antioxidant and anti-inflammatory effects and suggesting its potential role as adjuvant therapeutic alternatives in dealing with inflammatory-related diseases.

In the present work the genetic endpoint was also included by studying chromosomal deformities in the bone marrow and spermatocytes (somatic and germ cells). The results indicated that CCl_4 significantly induced chromosomal abnormalities in both somatic and germ cells. These results are in the same line with other authors (Dianovsk & Ivikova, 2001; Diab et al., 2018). CCEO at the highest tested dose was non-mutagenic. Such result was supported by other authors (Rabbani et al., 2005). Furthermore, CCEO displayed significant antigenotoxic effect against CCl_4-induced chromosomal damage in bone marrow and spermatocyte cells at the three dose levels (dose-dependently). The strongest activity was demonstrated at the highest tested dose (0.3mL/kg) where the inhibitory index values in the percentage of aberrant cells reached 62% and 55% in the bone marrow and spermatocyte cells respectively. It is worth mentioning that chromosomal damage is associated with many human diseases such as Alzheimer, cancer, aging, infertility, etc. (Tse et al., 2018; Barroso-Vilares & Logarinho, 2019 ; Muratori & De Geyter, 2019). Previous works supported the antimutagenic effect of *Cymbopogon citrates*. Meevatee et al. (1993) reported that lemongrass inhibited chromosome damage induced by mitomycin C in human lymphocytes while Bidinotto et al. (2011) demonstrated the protective role of lemongrass essential oil against N-methyl-N-nitrosurea (MNU) induced DNA damage in female Balb/C mice. Scavenging of ROS is considered the main mechanism for inhibiting the DNA damage induced by CCl_4 (Abdel-Moneim et al., 2017).

The results of the present work demonstrated the bio-safety of the essential oil of *Cymbopogon citrates* as evidenced by its normal effect on liver/kidney markers, oxidative stress markers and CAs analysis in bone marrow cells and in mouse spermatocytes compared to control. In addition to its hepat/renal and DNA protection. Lemongrass essential oil is a complex of many bioactive compounds. In the present work, a total of 12 constituents were identified by GC-MS. The major secondary metabolites of *Cymbopogon citrates* oil were in descending concentrations E-Citral; Geraniol; β-Myrcene; Nerol; Geranyl acetate; isoneral and linalool respectively representing 90.4 % of the total oil. These constituents possess different bioactive efficiency. Bayala et al. (2018) estimated the cytotoxic effect of *Cymbopogon citratus and Cymbopogon giganteus* essential oils on cancer cell lines and identified the antiproliferative effect of citral which is the major constituent of *Cymbopogon citrates.* In this study, the essential oil of *C. citratus* showed the more pronounced capability to scavenge $DPPH^+$ radicals (approximately 68% at 8 mg/mL). It was the most effective on prostate cancer cell lines PC-3 (IC_{50} = 32.1 µg/mL) and LNCaP (IC_{50} = 6.36 µg/mL), and on glioblastoma cell lines SF-763 (IC_{50} = 172.05 µg/mL) and SF-767 (IC_{50} = 45.13 µg/mL). In the above-mentioned study the activity of the essential oil of *C. citratus* was demonstrated to be equal to that of its major component citral. The authors suggested citral as a new and promising compound for the treatment of prostate cancer and glioblastoma. Zielińska et al. (2018) also demonstrated the anti-inflammatory and anticancer properties of citral. With GC/MS analysis geraniol represents the second major secondary metabolite in the oil of *C citratus*. Hasan & Sultana (2015) reported that geraniol alleviated 2-acetylaminofluorene-induced inflammation, apoptosis and oxidative stress in the liver of wistar rats. Radical scavenging activity and antioxidant capacity were reported for geraniol, β-myrcene and linalool (Jayaprakasha et al., 2012; Bentayeb et al., 2014; Noacco et al., 2018). Also, linalool possesses strong antitumorigenic potential in 180 solid tumor sarcoma model *in vivo* which is accompanied by modulation of oxidative stress (Jana et al., 2014). In this respect, the authors demonstrated the advantage of linalool which showed differential cytotoxicity towards tumor and normal cells in contrast to the anticancer drug cyclophosphamide, which is uniformly toxic to both. It was documented that the essential oils of various aromatic plant species, such as *Cymbopogon citratus* contain citronellol which is monoterpene alcohol. It was reported to have anti-inflammatory and antioxidant properties in rodents (Brito et al., 2012). *Cymbopogon citratus* essential oil is a complex of many phytochemical constituents which together play a critical role in the confirmed results concerning its hepatic/renal and DNA protective effect.

Conclusions

Going through the current results and discussion, it can be concluded that the present study is very encouraging, whereas it demonstrated the protective effect of the essential oil of *Cymbopogon citratus* on liver, kidney and DNA toxicities. Identification of its main phytochemicals by GC/MS analysis demonstrated the presence of 12 compounds, where E. Citral and Geraniol represent the major active constituents. The protective effect of CCEO together with its pleasant odor; suggesting that this oil could be a promising natural source in both pharmaceutical and food industry applications.

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