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Iso-caryophyllene cytotoxicity induced by lipid peroxidation and membrane permeabilization in L-929 cells

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ABSTRACT

Iso-caryophyllene, a sesquiterpene, is present in several essential oils from vegetable species. In previous work, iso-caryophyllene was found cytotoxic against *in vitro* culture cell lines but its mechanism of action is still unknown. Reactive oxygen species (ROS) and lipid oxidation induced by iso-caryophyllene were assessed using DCFH-DA and BODIPY-C11, respectively. The results show that iso-caryophyllene induces significant overproduction of ROS by about 187 % at 100 µM and lipid oxidation, which are both partially inhibited by α-tocopherol. The effect of isocaryophyllene on membrane permeabilization was evaluated using calcein-AM assay that show that iso-caryophyllene causes membrane permeabilization and cell shrinking. α-Tocopherol significantly prevents membrane permeabilization, cell shrinking and cell death, suggesting that lipid oxidation is in part implied in the cytotoxicity. Electrochemical experiments indicate that the superoxide anion reacts with iso-caryophyllene and possibly form oxidized derivatives which could initiate lipid oxidation. Interestingly, superoxide anion reacts more readily with α-tocopherol in comparison with iso-caryophyllene which could explain its protective effect on cellular membrane.

INTRODUCTION

Caryophyllenes, including β-caryophyllene, α-caryophyllene(α-humulene)andγ-caryophyllene(isocaryophyllene), are sesquiterpenes present in various essential oils such as *Eugenia caryophyllus*, *Humulus lupulus*, *Teucrium marum* and *Lantana achyranthifolia* (Katsiotis *et al*., 1990; Hernandez *et al*., 2005; Ricci *et al*., 2005; Jirovetz *et al*., 2006). Natural bicyclic β-caryophyllene and iso-caryophyllene are *trans* and *cis* double isomers, respectively, while α-humulene is a ring-opened isomer. In essential oils, β-caryophyllene is frequently found mixed with isocaryophyllene and/or α-humulene (Budavari, 1996). Iso-caryophyllene has been very seldom studied and its toxicity is still unknown. On the other hand, β-caryophyllene is much more known due to its use as a cosmetic ingredient and a food flavoring additive (Opdyke, 1973; de Groot *et al*., 1994; JECFA, 2006). Additionally, β-caryophyllene was used as skin penetration enhancers to promote the passage of compounds through the skin or cytoplasmic membrane (Cornwell and Barry, 1994; Legault and Pichette, 2007). Moreover, this molecule is known to possess anti-inflammatory (Tambe *et al* 1996; Cho *et al* 2007), anti-carcinogenic (Zheng *et al* 1992), antibiotic (Alma *et al*., 2003; Lourens *et al*., 2004; Pichette *et al*., 2006), antioxidant (Lourens *et al*., 2004; Singh *et al*., 2006) and local anaesthetic properties (Ghelardini *et al*., 2001). Recently, β-caryophyllene was reported as a selective CB2 agonist (Gertsch *et al*., 2008).

However, autoxidation of β-caryophyllene to caryophyllene oxide can cause allergic contact dermatitis (Sköld *et al*., 2006). The toxicity of β-caryophyllene has been well studied and it is relatively safe since it is not mutagenic and not carcinogenic (Seifried *et al*., 2006; Molina-Jasso *et al*., 2009) and was not cytotoxic in cell culture.

Surprisingly, iso-caryophyllene induces significant cytotoxicity in culture cell lines (Legault, *et al*., 2003). The mechanism of cytotoxicity of iso-caryophyllene is not well understood. In this study, the mechanism responsible of the cytotoxicity of iso-caryophyllene in L-929 cell lines will be investigated.

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MATERIAL AND METHODS

Chemicals

Iso-caryophyllene, dichlorofluorescin-diacetate (DCFH-DA), resazurine, Hoechst 33342, (±) -6-hydroxy-2,5,7,8-tetra methylchromane-2-carboxylic acid (Trolox), trypan blue, □-tocopherol, *tert*-butyl hydroperoxide (tBH), cumenehydroperoxide (CHP), diethyl maleate ester (DEM), sodium dodecyl sulfate (SDS), EDTA, tris-HCL, acetonitril, saponin, and sodium chloride were obtained from Sigma-Aldrich (Oakville, Canada). Monochlorobimane, BODIPY C_{11}, and iodurepropidium were obtained from Molecular Probe.

Calcein-AM was obtained from Calbiochem. Tetrabutylammonium tetrafluoroborate (TBA (BF_4)) was obtained from Fluka, and ethanol was obtained from Commercial Alcohols (Montréal, Québec).

Cell culture

L-929 cells (murinfibrosarcoma) were obtained from American Type Culture Collection (ATCC, Manassas, USA). Cells were grown, as describe by ATCC procedures in minimal essential medium (MEM) completed with 10 % foetal calf serum, 100X vitamins, 100X sodium pyruvate, 100X non essential amino acids, 100 IU penicillin and 100 µg mL^{-1} streptomycin. Cells were incubated at 37°C at a 5 % CO_2 humidified atmospheric conditions. In all cell based experiments, final solvent concentration was kept under 0.5 % (v/v) to avoid ethanol toxicity.

Cytotoxic assay

Cytotoxicity was evaluated using the resazurin reduction test (O'Brien *et al.*, 2000). Cytotoxicity was expressed in survival percentage in comparison with untreated cells. Fluorescence was measured using an automated Fluoroskan Ascent FL™ plate reader (Thermo Labsystems) with excitation wavelength of 530 nm and emission wavelength of 590 nm. To study antioxidant effects on cell survival, α-tocopherol (vitamin E) 200 µM and (±) -6-hydroxy-2,5,7,8-tetra methylchromane-2-carboxylic acid (Trolox) 100 µM were used.

Reactive oxygen species (ROS) quantification

Reactive oxygen species production was measured with 2,7-dichlorofluorescin-diacetate (DCFH-DA) (Wang and Joseph, 1999). L-929 cells were seeded in 96-wells microplates at $1x10^4$ cells per well in 100 µL of culture medium and incubated 24 hours at 37°C. Cells were then washed with 100 µL PBS 1X and incubated 1 hour at 37°C with 100 µL of HBSS 1X containing DCFH-DA 20 µM. After another wash with PBS 1X, cells were incubated again at 37°C with or without *tert*-butyl hydroperoxide (tBH) 200 µM (positive control), ethanol (solvent control), and iso-caryophyllene (400 µM to 50 µM), in presence or absence of an antioxidant. Fluorescence was measured using an automated Fluoroskan Ascent FL™ plate reader (Thermo Lab systems) with excitation wavelength of 485 nm and emission wavelength of 530 nm.

Lipid Peroxidation measurement

Lipid peroxidation was measured using BODIPY C_{11} (Molecular Probe), according to an adapted procedure (Drummen *et al*., 2004). L-929 cells were seeded in 96-wells microplates at $5x10^3$ cells per well in 100 µL of culture medium and incubated 24 hours at 37°C. Then, cells were washed with 100 µL of HBSS and incubated once again during 24 hours at 37°C with 100 µL of culture medium, with or without antioxidant. To study the effects of antioxidants on lipid peroxidation in presence of iso-caryophyllene, vitamin E (400 µM) and Trolox (100 µM) were used. After this second incubation, cells were washed with 100 µL HBSS and incubated 30 minutes at 37°C with BODIPY C_{11} (10 µM) in culture medium. After a second wash with 100 µL HBSS, cells were incubated at 37°C with HBSS, with or without ethanol (solvent control), iso-caryophyllene (400 µM to 50 µM), and cumenehydroperoxyde (CHP) 20 µM (positive control). Fluorescence was measured with an automated Varioskanplate reader (Thermo Electron Corporation) with an excitation wavelength of 590 nm and emission wavelength of 635 nm.

Membrane permeability measurement using calcein-AM

L-929 cells were seeded in 96-wells microplates at $1x10^4$ cells per well in 100 µL of culture medium and incubated at 37°C overnight. Cells were then washed with 100 µL MEM and incubated 90 minutes at 37°C with a calcein-AM solution (4 µM). Then, cells were washed with 100 µL MEM and incubated 90 minutes at 37°C with MEM, with or without ethanol (solvent control), iso-caryophyllene (400 µM to 50 µM) and saponin 2 % (positive control). Then, MEM was removed and replaced with fresh MEM. Fluorescence was measured with an automated Varioskan plate reader (Thermo Electron Corporation) with excitation wavelength of 480 nm and emission wavelength of 530 nm (Thorpe *et al.*, 1995).

Electrochemical measurements

All electrochemical experiments were carried out in anhydrous acetonitrile (ACN) (99.8 % (Sigma-Aldrich)) containing 0.1 M tetrabutylammonium tetrafluoroborate (TBA (BF_4)) (Fluka electrochemical grade (≥ 99 %)) as electrolyte. Prior to each experiment, tetrabutylammonium tetrafluoro-borate salt was allowed to dry at 115°± 3°C for two hours. Iso-caryophyllene (98 %) (Sigma-Aldrich) was used without further purification.

Cyclic voltammetry (CV) experiments were performed using a PAR 263-2A potentiostat. All measurements were performed in a 20 mL three-electrode electrochemical cell. A glassy carbon (GC) disk electrode with an area of 0.071 cm^2 was used as a working electrode. A platinized foil served as counter electrode and a Ag/Ag^+ electrode ($AgNO_3$ 0.01 M | TBA (BF_4) 0.1 M | ACN) as reference. The GC disk working electrode was polished using 0.05 µm alumina suspension on a polishing cloth before each experiment. All cyclic voltammograms were recorded at a controlled temperature of 20°± 0.2°C. The working electrode

was kept stationary and a potential sweep rate of 0.050 V s^{-1} was used. In order to investigate the reactivity of iso-caryophyllene toward superoxide ion, this reactive oxygen species (ROS) was directly electrogenerated *in situ* by the electrochemical reduction of dissolved O_2. Prior to the electrolysis, the reduction potential of O_2 was determined using cyclic voltammetry in O_2 saturated solution. Afterward, to quantitatively generate superoxide ion, the electrochemical reduction of O_2 was achieved at a constant potential, using a reticulated glassy carbon (RGC) electrode. The RGC electrode was used since it allows the massive production of O_2^- species within a short time because of its high specific area. Thereafter, the remaining dissolved O_2 was removed from the solution by a N_2 purging step. Finally, quantitative determination of iso-caryophyllene was done using GC electrode.

Statistical analysis

Data were analysed with Statview 5.0 (SAS Institute Inc.). Presented data are the mean of three measurements with standard deviation. Statistical comparison were performed using Kruskal-Wallis test and post hoc Student-Newman-Keuls test; $P \leq 0.05$. Each experiment was done at least three times.

RESULTS

Reactive oxygen species (ROS) overproduction induced by iso-caryophyllene was evaluated using DCFH-DA assay in L-929 cells. DCFH-DA crosses membrane and it is cleaved to non-fluorescent DCFH by intracellular esterase. DCFH can be oxidized to fluorescent DCF by various oxidants (Afri *et al*., 2004). L-929 cells were incubated for 48 hours with growing concentration of iso-caryophyllene ranging from 50 to 400 µM. The result presented in Fig. 1 shows that iso-caryophyllene induces significant oxidation of DCFH probes with an increasing of relative fluorescence by about 57, 187, 490 and 719 % at iso-caryophyllene concentrations of 50, 100, 200 and 400 µM, respectively. The effect of hydrophilic antioxidant Trolox (100 µM) was evaluated in L-929 cells treated with 50, 100, 200 and 400 µM iso-caryophyllene. Trolox does not inhibit ROS overproduction at doses of 50 and 100 µM of iso-caryophyllene. In contrast, at higher doses of 200 and 400 µM, Trolox inhibits DCFH oxidation induced by iso-caryophyllene by about 49 and 61 %, respectively. The effect of hydrophobic antioxidant α-tocopherol was also investigated. The results show that α-tocopherol (200 µM) significantly decreases iso-caryophyllene-induced DCFH oxidation to fluorescent DCF by about 58, 68, 74 and 69 % at concentrations of 50, 100, 200 and 400 µM, respectively.

Lipid peroxidation was evaluated using BODIPY-C_{11} assay. This highly hydrophobic fluorochrome inserts preferentially in membrane. It can react with ROS and become fluorescent. Therefore, BODIPY-C_{11} is a good marker to measure lipid peroxidation in membrane (Yoshida *et al*., 2003).As shown in Fig. 2, a dose-dependent lipid peroxidation was observed in presence of iso-caryophyllene, with a significant increase of 40, 71, 113 and 148 % at concentrations of 50, 100, 200 and 400 µM, respectively. It has been noticed also that α-tocopherol (200 µM) significantly decreased lipid peroxidation induced by iso-caryophyllene with a reduction of 33, 41, 42 and 36 % at iso-caryophyllene concentrations of 50, 100, 200 and 400 µM, respectively. No significant decrease was observed in the presence of 100 µM Trolox.

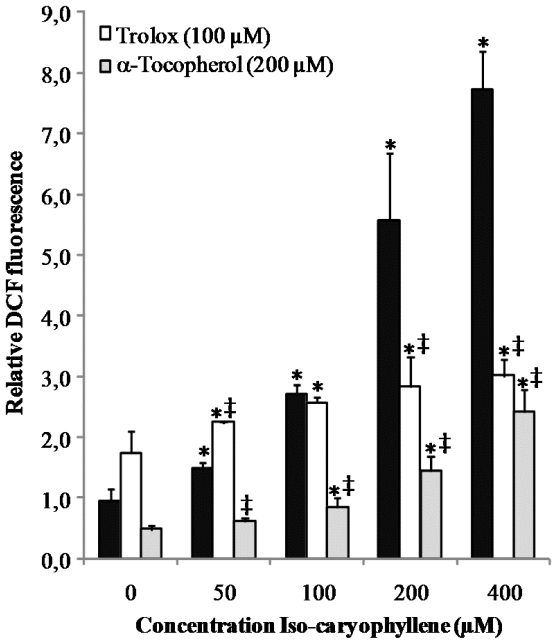

Fig. 1: Oxidative stress induced by iso-caryophyllene and effect of Trolox and α-tocopherol. Data represent the means ± standard deviation of three determinations. Significantly different from untreated cells; ‡Significantly different from iso-caryophyllene used alone; $P \leq 0.05$, Kruskal-Wallis test and post hoc Student-Newman-Keuls test.

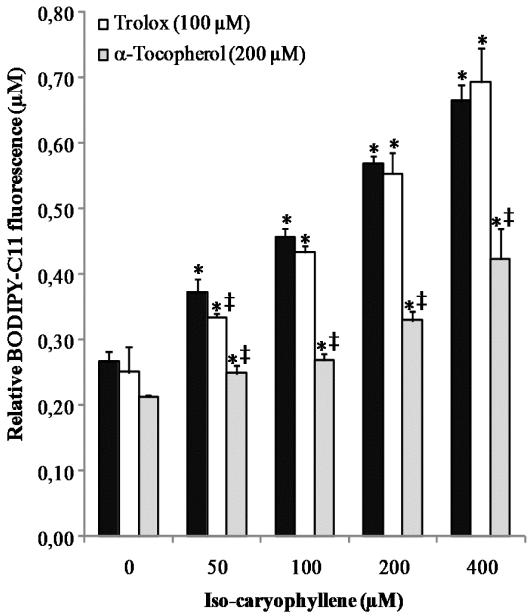

Fig. 2: Lipid peroxidation induced by iso-caryophyllene and effect of Trolox and α-tocopherol. Data represent the means ± standard deviation of three determinations. *Significantly different from untreated cells; ‡Significantly different from iso-caryophyllene used alone; $P \leq 0.05$, Kruskal-Wallis test and post hoc Student-Newman-Keuls test.

Membrane permeability in the presence of 50, 100, 200 and 400 µM iso-caryophyllene was evaluated using calcein-AM assay (Thorpe *et al*., 1995). This no fluorescent probe accumulates in the cells and the acetate group is cleaved by intracellular esterase. The fluorescent calcein cannot cross back the membrane and remains in the cell. When membrane permeability is altered, fluorescent calcein diffuses outside the cells and a partial loss of fluorescence can be observed (Hanning *et al*., 2000; Guzman and McCrae, 2005). Saponin was used as positive control with a decrease of calcein fluorescence of about 74 %. In Fig. 3, the results indicate that 200 and 400 µM iso-caryophyllene induce a significant membrane permeabilization. The decrease of calcein fluorescence is about 43 % and 57 % for iso-caryophyllene concentrations of 200 µM and 400 µM respectively. This observation was confirmed using propidium iodide probe (data not shown). The effect of antioxidant on membrane permeability was assessed in L-929 cells treated with iso-caryophyllene. In contrast to Trolox, the presence of 200 µM α-tocopherol almost completely prevents the decrease of calcein fluorescence induced by iso-caryophyllene 200 and 400 µM.

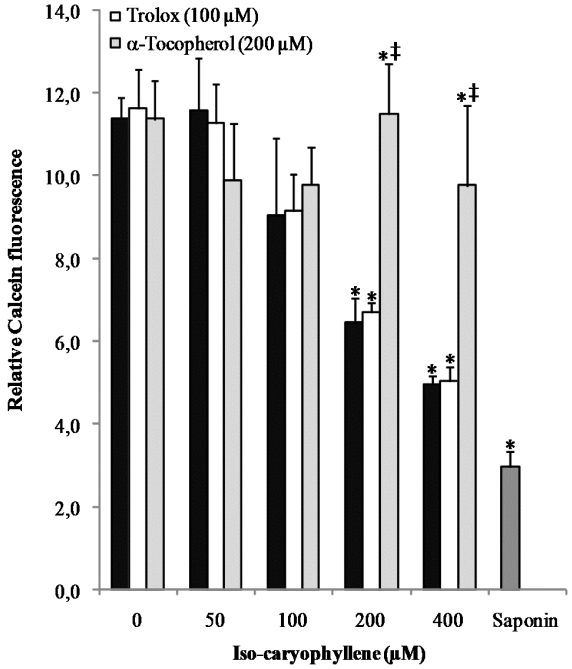

Fig. 3: Membrane permeabilization induced by iso-caryophyllene and effect of Trolox and α-tocopherol. Data represent the means ± standard deviation of three determinations. *Significantly different from untreated cells; ‡Significantly different from iso-caryophyllene used alone; P ≤ 0.05, Kruskal-Wallis test and post hoc Student-Newman-Keuls test.

To determine the cytotoxicity of iso-caryophyllene, L-929 cells were treated with increasing concentrations of iso-caryophyllene ranging from 6.25 to 400 µM for 48 h. The result presented in Fig. 4 shows that concentrations of iso-caryophyllene ranging from 6.25 to 50 µM do not significantly inhibit cell growth. However, iso-caryophyllene at concentrations of 100, 200 and 400 µM significantly decreases cell survival by about 54, 100 and 100 %, respectively. Trolox (100 µM) does not protect cells against isocaryophyllene cytotoxicity. However, α-tocopherol (200 µM) partially prevents the cytotoxicity of isocaryophyllene with a protective effect of about 25, 63 and 35 % at concentrations of 100, 200 and 400 µM, respectively.

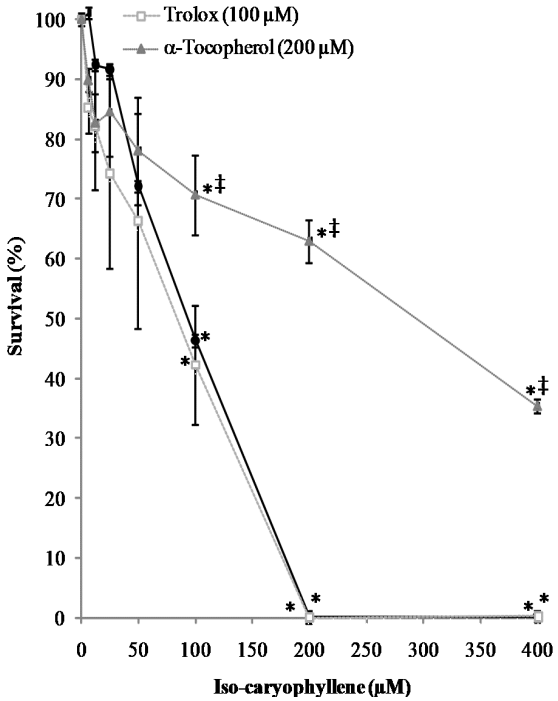

Fig. 4: Cytotoxicity induced by iso-caryophyllene and effect of Trolox and α-tocopherol. Data represent the means ± standard deviation of three determinations. *Significantly different from untreated cells; ‡Significantly different from iso-caryophyllene used alone; P ≤ 0.05, Kruskal-Wallis test and post hoc Student-Newman-Keuls test.

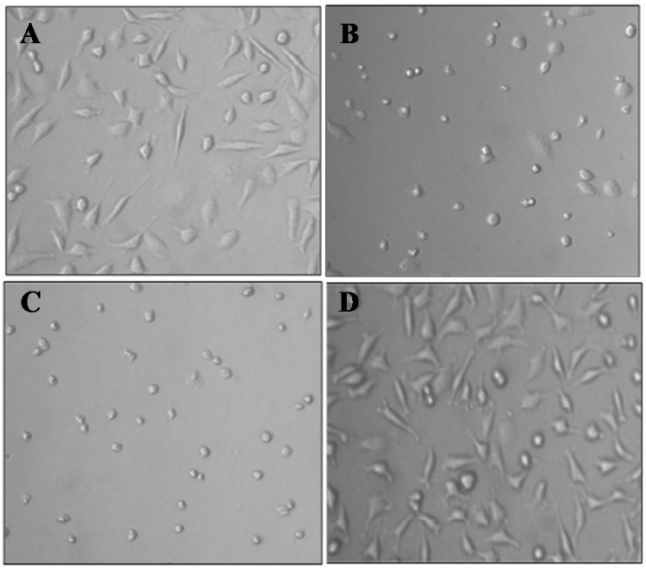

Fig. 5: Morphology of untreated L-929 cells (A) or treated with 200 µM iso-caryophyllene (B) ; 200 µM iso-caryophyllene with 100 µM Trolox (C) ; and 200 µM iso-caryophyllene with 200 µM α-tocopherol (D).

As shown in Fig. 5, the protective effect of α-tocopherol (200 µM) was confirmed by microscopic visualization of L-929 cells. In comparison with untreated cells (Fig. 5A), isocaryophyllene 200

µM induces cell shrinking characterized by cell rounding and an important decrease in their volume (Fig. 5B). No protective effect is noted when 100 µM Trolox was combined with 200 µM iso-caryophyllene (Fig. 5C). However, the presence of 200 µM α-tocopherol prevents cell shrinking induced by iso-caryophyllene (Fig. 5D).

To evaluate the propensity of iso-caryophyllene to be oxidized, its electrochemical behavior has been studied in anhydrous acetonitrile. Fig. 6 shows the voltammogram obtained in a iso-caryophyllene solution where electrochemical oxidation of this molecule is clearly observed by the presence of an anodic current peak having a maximum intensity at 1.35 V.

However, this oxidation is more difficult compared to the well-known antioxidant α–tocopherol (oxidation at 0.50 V, see Fig. 6).

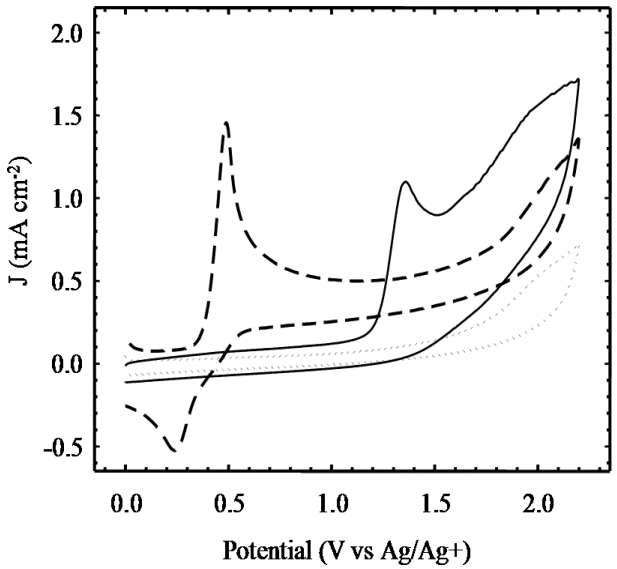

Fig. 6: Cyclic voltammogram at a glassy carbon disk electrode obtained in: acetonitrile + 0.1 M TBA (BF_4) solution (·····) ; acetonitrile + 0.1 M TBA (BF_4) + 3.0 mM α-Tocopherol) solution (- - - -) and acetonitrile + 0.1 M TBA (BF_4) + 2.2 mM iso-caryophyllene solution (——). d*E*/d*t* = 0.050 V s^{-1}.

While iso-caryophyllene does not have a strong antioxidant character like α–tocopherol, iso-caryophyllene can be oxidized electrochemically and its oxidation by a chemical oxidant such as the superoxide anion ($O_2^{\cdot-}$) could be envisaged.

This anion can be electrogenerated*in situ* (electrochemical reduction) on a glassy carbon electrode using dissolved oxygen in acetonitrile, see Fig. 7A. By exposing iso-caryophyllene to electrogenerated $O_2^{\cdot-}$ and by monitoring its quantity by the analysis of the current peak intensities, iso-caryophyllene reactivity toward $O_2^{\cdot-}$ could be assessed.

Figure 7B displays the voltammogram of iso-caryophyllene solution after exposure to various amounts of electrogenerated $O_2^{\cdot-}$. It was observed that the oxidation current peak present at 1.35 V disappears gradually as the electrogenerated $O_2^{\cdot-}$ amount increases confirming that the iso-caryophyllene molecules are consumed in the presence of $O_2^{\cdot-}$.

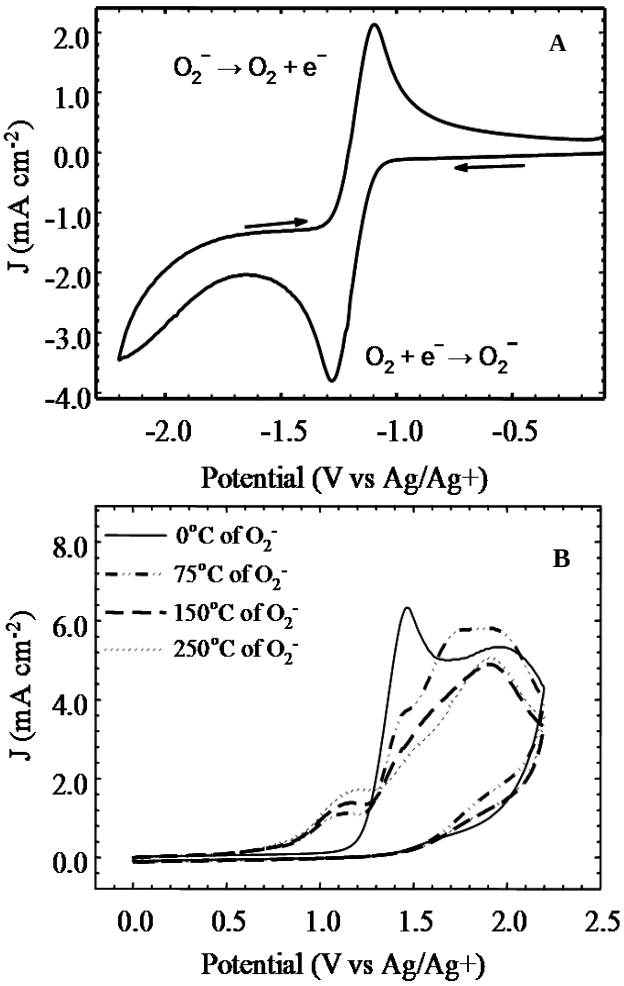

Fig. 7: Cyclic voltammograms at a glassy carbon disk electrode obtained in an acetonitrile solution with 0.1 M TBA (BF_4) and saturated with oxygen. d*E*/d*t* = 0.050 V s^{-1} (A) or with 0.1 M TBA (BF_4) + 17.5 mM iso-caryophyllene solution. d*E*/d*t* = 0.050 V s^{-1} (B).

DISCUSSION

In previous studies, the cytotoxic humulene was found to induce oxidative stress in culture cell lines (Legault *et al*., 2003). Therefore, evaluation of the effect of iso-caryophyllene on ROS overproduction in L-929 cell lines using DCFH probes was performed. DCFH can accumulate inside cytoplasm and also in the cytoplasmic membrane (Yoshida *et al*., 2003; Afri *et al*., 2004). Consequently, it is difficult to distinguish between an oxidation of the membrane and a cytoplasmic oxidation. It was reported that hydrophobic antioxidant as α-tocopherol was more effective to inhibit lipid oxidation in comparison with hydrophilic Trolox (Massey and Burton, 1990; Kaneko *et al*., 1991; Inar *et al*., 2006). Therefore, evaluation of the protective effect of Trolox and α-tocopherol on the ROS overproduction induced by iso-caryophyllene was also performed to determine roughly the subcellular site of oxidation. The results indicate that iso-caryophyllene strongly induces overproduction of ROS which is significantly suppressed by α-tocopherol. In constrast, Trolox inhibit ROS only at high doses of iso-caryophyllene. Overall, these

results suggest that iso-caryophyllene possibly induces lipid oxidation of cellular membrane. The oxidation of BODIPY-C11 induced by iso-caryophyllene, a probe measuring specifically lipid oxidation, supports this hypothesis (Yoshida *et al*., 2003). As expected, α-tocopherol inhibits BODIPY-C11 oxidation induced by iso-caryophyllene but not Trolox. Lipid oxidation was reported to alter membrane permeability and induce cell death (Stark, 2005; Pamplona, 2008). Iso-caryophyllene causes membrane permeabilization and cell shrinking, which are inhibited by α-tocopherol. Moreover, α-tocopherol protects against cytotoxicity of iso-caryophyllene in L-929 cells but not Trolox. Altogether, these results strongly suggest that the cytotoxicity of iso-caryophyllene is induced by lipid oxidation, membrane permeabilization and cell shrinking. Caryophyllene oxide was reported to inhibit mitochondrial electron transport chain via direct complex I inhibition (Monzote *et al*., 2009). Therefore, like caryophyllene oxide, it is possible that iso-caryophyllene also blocks mitochondrial electron transport chain generating ROS such as superoxide anion and hydrogen peroxide (Fariss *et al*., 2005).

Additionally, the reactivity of iso-caryophyllene with superoxide anion was evaluated using electrochemical oxidation and compared with α-tocopherol. Clearly, the results show that iso-caryophyllene can react with superoxide anion. Therefore, iso-caryophyllene oxidized derivatives formed in the cell membrane could possibly initiate lipid peroxidation. This assumption will be verified in a cellular model. Moreover, superoxide anion reacts more easily with α-tocopherol than iso-caryophyllene. These results could explain, in part, the protective effect of α-tocopherol. Lipid peroxidation products were found mutagen and carcinogen, consequently, iso-caryophyllene may be genotoxic (Hu *et al*., 2002; Niedernhofer, 2002; Feng *et al*., 2003). To the best of our knowledge, the genotoxicity of the iso-caryophyllene is unknown and it would be appropriate to evaluate this possibility. Surprisingly, trans-caryophyllene (β-caryophyllene) was not cytotoxic and not genotoxic suggesting that the endocyclic double bond is less reactive or more stable than the exocyclic double bond (iso-caryophyllene) but this hypothesis needs more investigations.

CONCLUSION

In conclusion, the results presented in this work show that cytotoxicity of iso-caryophyllene is induced by lipid oxidation, membrane permeabilization and cell shrinking. α-Tocopherol protects the cell against cytotoxicity inhibiting lipid oxidation and membrane permeabilization. Lipid oxidation could be initiated by oxidized iso-caryophyllene derivatives generated by superoxide anion.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Afri M, Frimer AA, Cohen Y. Active oxygen chemistry within the liposomal bilayer part IV : locating 2',7'-dichlorofluorescein (DCF, 2'-7'-dichlorodihydrofluorescein (DCFH) and 2'7'-dichlorodihydrofluorescein diacetate (DCFH-DA) in the lipid bilayer. Chem. Phys. Lipids. 2004; 131:123–133.

Alma MH, Mavi A, Yildirim A, Digrak M, Hirata T. Screening chemical composition and *in vitro* antioxidant and antibacterial activities of the essential oils from Origanumsyriacum L. growing in Turkey. Biol. Pharm. Bull. 2003; 26:1725–1729.

Budavari S (Ed.). The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals, 12th ed. Merck & Co Inc., New Jersey, (1996) 308.

Cho JA, Chang HJ, Lee S-K, Kim H-J, Hwang J-K, Chun HS. Amelioration of dextran sulphate sodium-induced colitis in mice by oral administration of β-caryophyllene, a sesquiterpene. Life Sci. 2007; 80:932–939.

Cornwell PA, Barry BW.Sesquiterpene components of volatile oil as skin penetration enhancers for the hydrophilic permeant 5-fluorouracil. J. Pharm. Pharmacol. 1994; 46:261–269.

de Groot AC, Weyland JW, Nater JP. Tabulations of ingredients of cosmetics. In: de Groot, A.C., Weyland, J.W., Nater, J.P. (Eds.), Unwanted Effects of Cosmetics and Drugs used in Dermatology. Elsevier, Amsterdam, (1994) 579.

Drummen GP, Makkinje M, Verkley AJ, Op den Kamp J.A., Post J.A,. Attenuation of lipid peroxidation by antioxidants in rat-1 fibroblasts comparison of the lipid peroxidation reporter molecules cis-parinaric acid and C-11 BODIPY (581/591) in a biological setting. Biochim. Biophys. Acta. 2004; 1636:136–150.

Fariss MW, Chan CB, Patel M, Houten BV, Orrenius S. Role of mitochondria in toxic oxidative stress. Mol. Interventions. 2005; 5:94–111.

Feng Z, Hu W, Amin S, Tang M-S. Mutational spectrum and genotoxicity of the major lipid peroxidation product, trans-4-hydroxy-2-nonenal, induced DNA adducts in nucleotide excision repair-proficient and -deficient human cells. Biochemistry. 2003; 42:7848–7854.

Gertsch J, Leonti M, Raduner S, Racz I, Chen J-K, Xie X-Q, Altmann K-H,

Karsak M, Zimmer A. Beta-caryophyllene is a dietary cannabinoid. Proc. Natl. Acad. Sci. 2008; 105:9099–9104.

Ghelardini C, Galeotti N, Di Cesare M-L, Mazzanti G, Bartolini A. Local anaesthetic activity of β-caryophyllene. Farmaco. 2001; 56:387–389

Guzman E, McCrae MA. A rapid and accurate assay for assessing the cytotoxicity of viral proteins. J. Virol. Methods. 2005; 127:119–125.

Hanning J, Zhang D, Canaday D J, Beckett MA, Astumian RD, Weichsekbaum RR, Lee RC. Surfactant sealing of membranes permeabilized by ionizing radiation. Radiat. Res. 2000; 154:171–177.

Hernandez T, Canales M, Avila JG, Garcia AM, Martinez A, Caballero J, Romo de Vivar A, Lira R. Composition and antibacterial activity of essential oil of *Lantana achyranthifolia*Desf. (Verbenaceae). J Ethnopharmacol. 2005; 96:551–554.

Hu W, Feng Z, Eveleigh J, Iyer G, Pan J, Amin S, Chung F-L, Tang M-S. The major lipid peroxidation product, trans-4-hydroxy-2-nonenal, preferentially forms DNA adducts at codon 249 of human p53 gene, a unique mutational hotspot in hepatocellular carcinoma. Carcinogenesis. 2002; 23:1781–1789.

Inar A, Rogero MM, Junqueira RB, Carrapeiro MM. Free radical scavenger and antioxidant capacity correlation of α-tocopherol and

Trolox measured by three in vitro methodologies. Int. J. Food Sci. Nutr. 2006; 57:75–82.

JECFA. 2006. WHO Food Additive Series 54: Aliphatic and Alicyclic Hydrocarbons.

Jirovetz L, Buchbauer G, Stoilova I, Stoyanova A, Krastanov A, Schmidt E. Chemical composition and antioxidant properties of clove leaf essential oil. J. Agric. Food Chem. 2006; 54:6303–6307.

Kaneko T, Nakano S-I, Matsuo M. Protective effect of vitamin E on linoleic acid hydroperoxide-induced injury to human endothelail cells. Lipids. 1991; 26:345–348.

Katsiotis ST, Langezaal CR, Scheffer JJC. Composition of the essential oils from leaves of various *Humulus lupulus*L. Cultivars. Flavour Frag. J. 1990; 5:97–100.

Legault J, Dahl W, Debiton E, Pichette A, Madelmont J-C,. Antitumor activity of balsam fir oil: production of reactive oxygen species induced by α-humulene as possible mechanism of action. Planta Med. 2003; 69:402–407.

Legault J, Pichette A. Potentiating effect of β-caryophyllene on anticancer activity of α-humulene, isocaryophyllene and paclitaxel. J. Pharm. Pharmacol. 2007; 59:1643–1647.

Lourens AC, Reddy D, Baser KH, Viljoen AM, Van Vuuren SF. In vitro biological activity and essential oil composition of four indigenous South African Helichrysum species. J Ethnopharmacol. 2004; 95:253–258.

Massey KD, Burton KP. Free radical damage in neonatal rat cardiac myocyte cultures: Effects of α-tocopherol, trolox, and phytol. Free Radical Biol. Med. 1990; 8:449–458.

Molina-Jasso D, Alvarez-González I, Madrigal-Bujaidar E.Clastogenicity of beta-caryophyllene in mouse.Biol. Pharm. Bull. 2009; 32:520–522.

Monzote L, Stamberg W, Staniek K, Gille L. Toxic effects of carvacrol, caryophyllene oxide, and ascaridole from essential oil of Chenopodiumambrosioides on mitochondria. Toxicol. Appl. Pharmacol. 2009; 240:337-347.

Niedernhofer LJ, Daniels JS, Rouzer CA, Greene RE, Marnett LJ. Malondialdehyde, a product of lipid peroxidation, is mutagenic in human cells. J. Biol. Chem. 2003; 278:31426–31433.

O'Brien J, Wilson I, Orton T,Pognan F. Investigation of the alamar blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity. Eur. J. Biochem. 2000; 267:5421–5426.

Opdyke DLJ. Monographs on fragrance raw materials. Caryophyllene, Fd Cosmet Toxicol. 1973; 11:1059–1060.

Pamplona R. Membrane phospholipids, lipoxidative damage and molecular intergrity: a causal role in aging and longevity. Biochim. Biophys. Acta. 2008; 1777:1249–1262.

Pichette A, Larouche P-L, Lebrun M, Legault J. Composition and antibacterial activity of *Abiesbalsamea* essential oil. Phytother. Res. 2006; 20:371–373.

Ricci D, Fraternal D, Giamperi L, Bucchini A, Epifano F, Burini G, Curini M. Chemical composition, antimicrobial and antioxydant activity of the essential oil of *Teucrium marum* (Lamiaceae). J Ethnopharmacol. 2005; 98:195–200.

Seifried HE, Seifried RM, Clarke JJ, Junghans TB, San RHC. A compilation of two decades of mutagenicity test results with the Ames *Salmonella typhimurium*and L5178Y mouse lymphoma cell mutation assays. Chem. Res. Toxicol. 2006; 19:627–644.

Singh G, Marimuthu P, de Heluani CS, Catalan CA. Antioxidant and biocidal activities of Carumnigrum (seed) essential oil, oleoresin, and their selected components. J. Agric. Food Chem. 2006; 54:174–181.

Sköld M, Karlberg A-T, Matura M, Börje A. The fragrance chemical β-caryophyllene–air oxidation and skin sensitization. Food Chem. Toxicol. 2006; 44:538–545.

Stark G. Functional consequence of oxidative membrane damage. J. Membr. Biol. 2005; 205:1–16.

Tambe Y, Tsujiuchi H, Honda G, Ikeshiro Y, Tanaka S. Gastric cytoprotection of the non-steroidal anti-inflammatory sesquiterpene, β-caryophyllene. Planta Med. 1996; 62:469–470.

Thorpe WP, Toner M, Ezzell RM, Tompkins RG, Yarmush ML. Dynamics of Photoinduced Cell Plasma Membrane Injury. Biophys. J. 1995; 68:2198–2206.

Wang H, Joseph JA. Quantifying cellular oxidative stress by dichlorofluoroscein assay using microplate reader. Free Radical Biol. Med. 1999; 27:612–616.

Yoshida Y, Shimakawa S, Itoh N, Niki E. Action of DCFH and Bodipy as a probe for radical oxidation in hydrophilic and lipophilic domain. Free Radical Res.2003; 37:861–872.

Zheng GQ, Kenney PM, Lam LK. Sesquiterpenes from clove (Eugenia caryophyllata) as potential anticarcinogenic agents. J. Nat. Prod.1992; 55:999–1003.

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