Change in Caco-2 cells following treatment with various lavender essential oils

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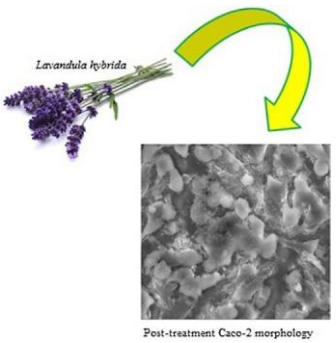
ABSTRACT

Lavender is an aromatic evergreen shrub diffused in the Mediterranean basin appreciated since antiquity. The genus *Lavandula* is part of Lamiaceae family and includes more than 20 species, among which true lavender (*L. vera* D.C. or *L. angustifolia* Miller.) and spike lavender (*L. latifolia* Medikus); there are also numerous hybrids known as lavandins (*L. hybrida* Rev.). *L. vera*, spike lavender and several hybrids are the most intensely used breeding species for the production of essential oils. Lavender and lavandin essential oils have been applied in food, pharmaceutical and other agro industries as biological products. In their chemical composition, terpenes linalool and linalyl acetate along with terpenoids such as 1,8-cineole are mostly responsible for biological and therapeutic activities. This study evaluates cytotoxic activity of essential oils derived from four lavender species on human epithelial colorectal adenocarcinoma cells. Analysis of pre- and post- treatment cell morphology has been performed using scanning electron microscope.



Post-treatment Caco-2 morphology

1. Introduction

Phytotherapy is one of the most antique practices used by human to cure health problems; its traces have been found among ancient civilisations including Egypt, Indus Valley, Greek, Chinese and Roman Empire (Campanini 2004). Lavender essential oils (EO) present important pharmacological properties and low toxicity, and are promising candidates to be used as food supplements or in pharmaceutical applications.

Even though lavender's antiseptic activity is less pronounced compared to that of EO derived from other plants of the Lamiaceae family such as thyme, it presents important anti-inflammatory and decongestant effects without causing irritations. These features make lavender EO extremely useful as a treatment of low-grade inflammations. While numerous scientific studies describe the composition and antimicrobial activity of EO extracted from multiple lavender species and hybrids, the action on human epithelial colorectal adenocar- cinoma (Caco-2) cells is poorly reported.

The study reported here presents a comparative screening of four medicinal plants includ- ing three different samples of *L. hybrida* and one sample of *L. vera* cv Selection, cultivated at the Herb Garden of Casola Valsenio (Emilia Romagna region, northern Italy). Oxygenated monoterpenes, such as 1,8-cineole, lavandulol and necrodane derivatives, are the main components of EO. They present a significant antioxidant activity with a high ability to inhibit lipid peroxidation and showed an outstanding effect against a wide spectrum of microorganisms including Gram positive and Gram negative bacteria, and pathogenic yeasts (Ait Said et al. 2015) (Cavanagh & Wilkinson 2002). Moreover, we analysed the cytotoxic activity of the four EOs on Caco-2 cells by the evaluation of cell morphology before and after treatment with the aid of scanning electron microscope.

2. Results and discussion

In this study we analysed four lavender EOs on Caco-2 cells. The EOs tested showed no cytotoxic effect at very low concentrations, ranging from 0.03% (IC50 0.9132 mg/mL) (*L. hybrid Rev*), 0.015% (IC50 0.7798 mg/mL) (*L. latifolia Medikus*), 0.008% (IC50 1.224 mg/mL) (*L. vera D.C.*), to 0.001% (IC50 1.631 mg/mL) (*L. angustifolia Miller*). The finding that lavender essential oil is a medicinal plant-derived natural multicomponent preparation may be a source of pharmacological active substances that interfere with Caco-2 cells. EOs are considered more potent than their constituents (Table S1) due to their synergistic and more selective effect. In addition, EOs from plants growing in varied environments differ in their composition and hence have different uses (Prusinowska et al. 2016) (Carrasco et al. 2016). Microscopic evaluation of cell monolayers highlighted their heterogeneous morphology confirmed even by visual inspection. As a result, over the years, the characteristics of the cells used in different laboratories around the world have diverged significantly, which makes it difficult to compare results across laboratories (Sambuy et al. 2005). The SEM appearance of tumor cells in the control group (Figure S1 (a)) showed well-preserved cells forming a monolayer (Meunier et al. 1995) and (Hidalgo et al. 1989). (Figure S1 (b)) showed Caco-2 cells treated with Lavandula hybrida at a non-cytotoxic concentration of 0.0005%. Caco-2 cells express tight junctions, microvilli and a number of enzymes and transporters characteristic of such enterocytes: peptidases, esterases, P-glycoprotein, uptake transporters for amino acids, bile acids carboxylic acids, etc. They appear flat, of variable and mostly lengthened shape, closely collocated with a narrow intercellular space however a few rounded cells, probably detached from the monolayer, were identified. Smooth surface with tiny microvilli, thin filamentous pattern inside the cells and wide nuclear relief were observed. Groups treated with cytotoxic EO concentrations presented the loss of uniform cell monolayer with wide empty spaces and few cell islands (Figure S1(c)). In the group where EO showed more cytotoxicity towards Caco-2 cells changes in cell morphology were observed (Figure S1(d)). The cells appeared bulgy and separated by wide intercellular spaces, with cell surface smoothened following the loss of microvilli and irregular due to the presence of numerous blebs. Moreover, cell debris was randomly revealed between cells (Gesi et al. 1998). These phenomena probably result from apoptosis induced by lipophilic compounds such as terpenes. The composition of lavender EOs suggest (Table S1) that *L. angustifolia Miller* has a high cytotoxicity, due to the large presence of

terpenes that cause apoptotic mechanisms. The anti-cancer activity of plant essential oils has been mostly ascribed to terpenoids as the majors compomponds. Also, many of isolated terpenoids have been shown to possess anti- proliferative and chemopreventive activities in various models (Kuttan et al. 2011).

EOs probably affect cell membrane permeability and act on different cellular targets involved in various pathways. EOs increase intracellular ROS/RNS levels which results in apoptosis in cancer cells. Inhibition of Akt, mTOR and MAPK pathways at different steps by EOs leads to corresponding up-/downregulation of various key biomolecules. Alteration in expression of NF-κB caused by exposure to EOs and further binding of NF-kB to DNA result as well in apoptosis in Caco-2 cells. Yet, dephosphorylation of Akt by the action of EOs leads to overexpression of p21, which either induces apoptosis by increasing caspases level or results in cell cycle arrest by binding to cyclins. In addition, EOs-induced mitochondrial stress leads to activation of Bcl-2 and membrane depolarization resulting in enhanced release of cytochrome-C to the cytoplasm, which activates apoptotic cell death. EOs also modulate DNA repair mechanisms by acting as DNA polymerase inhibitors and lead to PARP cleavage which also results in apoptosis in cancer cells (Gautam et al. 2014). It is plausible that the action of EOs determines minor cytotoxic effect as the cells represented in (Figure S1(c)) seem to have undergone less damage. In fact, they are visibly smaller, flattened due to the loss of nuclear relief, characterised by cell surface covered by thick microvilli and tend to assume rounded shape. When lower doses of EOs were applied, damaging effect on Caco-2 cells was only modest; cell monolayer presented a few empty spaces (Figure S1(e)), while the cells appeared flattened and outdistanced ones from the others (Figure S1(f)).

In eukaryotic cells, EOs change the fluidity of membranes, which become abnormally permeable resulting in leakage of radicals, cytochrome C, calcium ions and proteins, as in the case of oxidative stress and bioenergetic failure (Alizadeh & Aghaee 2016) (Yoon et al. 2000) (Armstrong 2006). Cytotoxic activities of EOs or their major components, sometimes activated by light, have been also demonstrated in mammalian cells *in vitro* by short-term viability assays using specific cell staining including MTT (3-(45 dimethylthiazol-2-yl)-25-di- phenyl-tetrazolium bromide) Test (Carvalho de Sousa et al. 2004). EO’s cytotoxicity in mam- malian cells is caused by induction of apoptosis and necrosis.

3. Conclusions

The present study was designed to evaluate the effect of EOs of lavender on cancer cell lines to see if these cells were suitable for treatment with natural products. Caco-2 cell line showed different susceptibility. Data presented here are related to a preliminary study; further study is necessary to understand the mechanism. Lavender EO components as linalool and linalyl acetate can induce cell death (Table S1). Although further work is needed for more elucida- tion, these preliminary data show that lavander EO could be developed as therapeutic agent on cancer cell lines. Further research is needed to clarify other relevant activities and to confirm '*in vivo'* our findings.

Disclosure statement

No potential conflict of interest was reported by the authors.

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