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Comparative Cytotoxic Effect Of Methanolic Extract Of *Cinnamon Zeylanicum* Bark With Commercial Trans Cinnamaldehyde In Animal Cell Culture

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Abstract:

A comparison of the cytotoxic effects of methanolic cinnamon extract (MCE) from the bark of Cinnamon zeylanicum L. (Lauraceae) and commercially available cinnamaldehyde was carried out using mouse cell line (C2C12). The bark of Cinnamon zeylanicum was extracted in 50% methanol and trans cinnamaldehyde was procured commercially. Methanolic cinnamon extract (MCE) is highly concentrated as a result showing 50% cell cytotoxicity to C2C12 cells at concentration 100 µg/ml than commercial cinnamaldehyde at 66.08 µg/ml.

Introduction

Cinnamon zeylanicum L. (Lauraceae) is thought to have originated in Sri Lanka and India's Malabar coast (Radhakrishnan *et al.*, 1992). Since ancient times, this plant has been utilised for a variety of uses, and its leaves and bark are employed in a variety of dietary applications (Jirovetz *et al.*, 1997). It has recently been demonstrated that the essential oil of *Cinnamon zeylanicum* from Cameroon has antiradical and antifungal properties against several common fungi that cause deterioration of stored food goods (Dongmo *et al.*, 2007). Cinnamon, a widely used food spice, has been shown to exhibit diverse biological functions including anti-inflammatory (Nikolić *et al.*, 2014), anti-oxidant (Wijesekera *et al.*, 1974; Severino *et al.*, 2015), anti-microbial (Jayatilaka *et al.*, 1995; Severino *et al.*, 2015), and anti-diabetic effects (Yu & Jang, 2007; Jantan *et al.*, 2008; Vangalapati *et al.*, 2012). Recently, the anti-tumor activity of cinnamon has been shown both in vitro (Prieto *et al.*, 1999; Ranasinghe *et al.*, 2013; Rao & Gan, 2014) and in vivo (Balti *et al.*, 2018).

Cinnamaldehyde is one of the most active and widely used flavoring agent obtained from *Cinnamon zeylanicum* and other spices of Cinnamon (Zhou *et al.*, 2015). About 98% of oil extracted from Cinnamon Bark consists of cinnamaldehyde. At first, it was isolated by Dumas and Péligot and then synthesized by Luigi Chiozza in his laboratory for the first time (Chiozza *et al.*, 1856). It naturally exists in trans confirmation (Zhang *et al.*, 2015). A bioavailability of 20 % was reported and it was found that its metabolic profile and excretion pattern are not affected by sex, dose size or route of administration. Cinnamaldehyde has been demonstrated to decrease the growth of various human cancer cell lines, including breast, leukaemia, ovarian, and lung cancer cells (Lee *et al.*, 1999).

In present study we have studied a comparative analysis of cytotoxic effect of methanolic extract of cinnamon (MCE) from *C. zeylanicum* with that of commercial cinnamaldehyde on C2C12 cell lines.

Materials and methods

Plant materials

FSTL (Flavourit Spices Trading Limited), Cochin, Kerala, India, provided the *Cinnamon zeylanicum* bark. Bark was dried in an oven at 37°C until it reached a consistent weight.

Preparation of *Cinnamon zeylanicum* extract

Cinnamon zeylanicum (30g) was coarsely crushed and extracted three times with 50% methanol (1g/10 ml) at room temperature in a shaker for 24 h, and supernatant pooled. After passing through a double-layered muslin cloth, the extract was centrifuged at 3000g for 5 minutes. To get the crude dry extract MCE, the solvent in the supernatant was evaporated at 40^{O} C in a rotary evaporator. Prior to dosing the cells, the extract was filter sterilized (pore size, 0.2µm) (Chaudhary *et al.,* 2020).

Cell culture and differentiation

C2C12 myoblast cells were cultured as described previous reports of the lab (Dutt *et al.*, 2018). Briefly, C2C12 myoblasts were proliferated in 48 well plate using Dulbecco`s modified eagle medium (DMEM) supplemented with 20% fetal bovine serum (FBS), 2.5 µg/ml amphotericin-B and 5 µg/ml ciprofloxacin in presence of 5% CO_2 at 37ºC.

Cell viability analysis (MTT assay)

The MTT assay was performed to determine the cytotoxic effect of MCE and Trans cinnamaldehye on proliferation rate of C2C12 myoblast cells (Dutt *et al.*, 2018). MTT test is a colorimetric assay that quantifies cell metabolic activity as a marker of cell viability and proliferation. SDH, a mitochondrial enzyme catalyzes the reduction of a tetrazolium salt (MTT-yellow in colour) to purple formazan crystals.

The MTT test is used to calculate the inhibitory concentration (IC_{50}), which is the concentration of any substance or medicine that allows 50 % of cells to survive. According to the procedure, 15,000 cells/well were seeded in 48 well plates and cultured in a CO_2 incubator for 24 h. They were treated with varied doses of extract and 0.1 % DMSO as a control after 24 h. GM - 20 was aspirated, and then washed with 300 µl PBS to fully remove the media contents. MTT (5 mg/ml) solution in DMEM without phenol red was put to each well of the plate containing cells and incubated for 3 - 4 h in a CO_2 incubator for formazan formation. To avoid cell detachment during incubation, DMEM without phenol red was employed. After aspirating MTT solution from each well, 250 µl DMSO was added to dissolve the formazan. After 2 h, the cells were collected, centrifuged, and the purple-colored solution was spectrophotometrically read at 570 nm and 690 nm. The % cell viability was computed with reference to the control, and a graph was drawn to show the relationship between cinnamon extract concentration and cell viability (Dutt *et al*., 2018).

Statistical analysis

Data for all parameters are expressed as mean ± standard deviation (SD). For confirmation of results, each experiment was performed multiple times independently. The results were subjected to one-way ANOVA test for statistical significance among experimental groups using SPSS (version 16.0) software. Results were considered significant at p value ≤ 0.05.

Results and discussion

Effect of methanolic cinnamon extract (MCE) from bark of *C. zeylanicum* on viability of C2C12 cell lines

The cytotoxicity of methanolic cinnamon extract (MCE) from bark of *C. zeylanicum* was evaluated on C2C12 myoblasts using the MTT assay. The obtained data revealed that at a higher concentration of 500 µg/ml, MCE induced nearly 90% cell death and significantly altered the morphology of myoblasts, indicating a cytotoxic effect at this concentration. However, at a concentration of 100 µg/ml, MCE resulted in 50% cell death without causing any changes in myoblast morphology. This led us to conclude that the IC_{50} of MCE is 100 µg/ml.

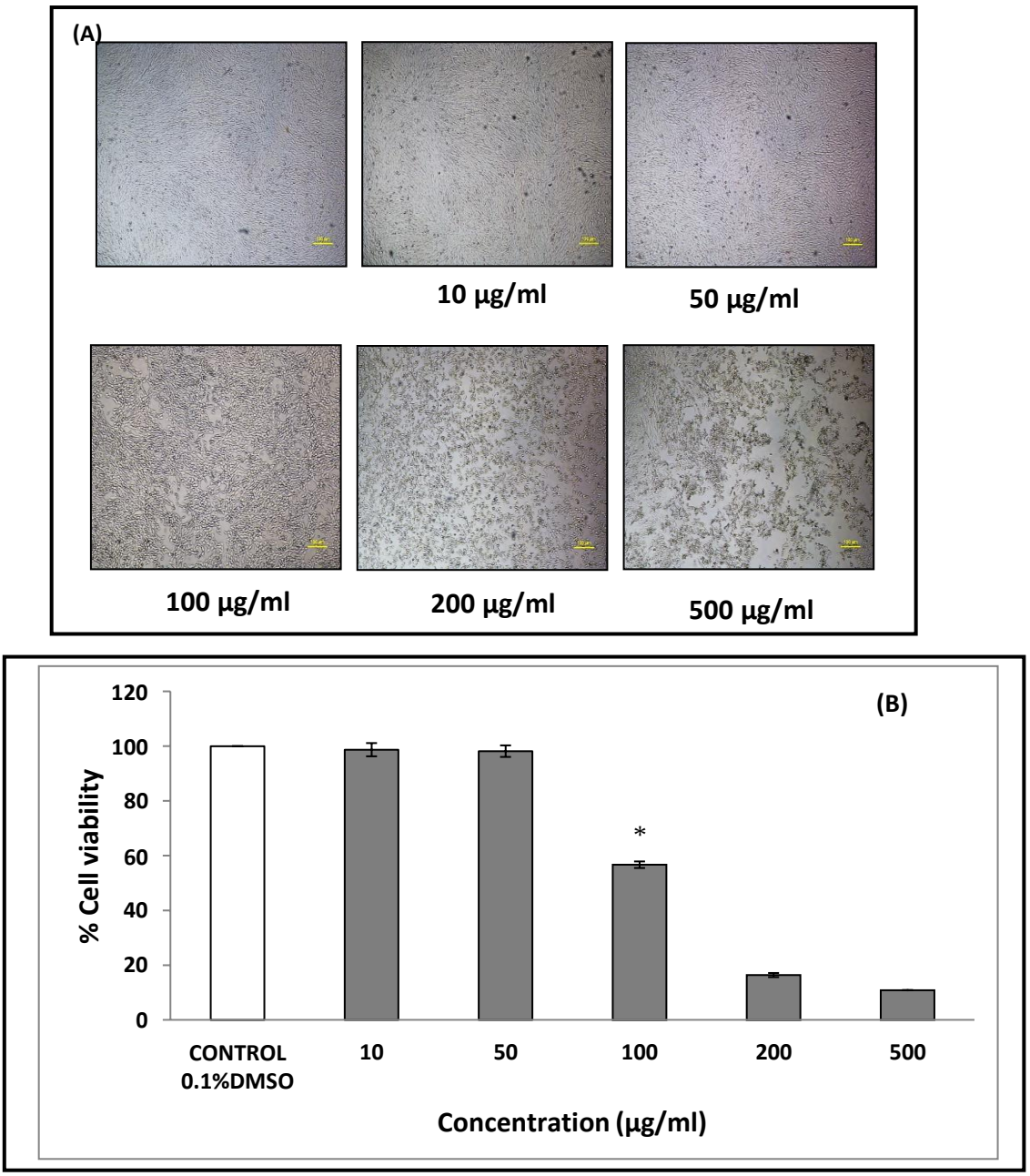

Fig 1: Effect of methanolic cinnamon extract (MCE) on C2C12 cells: **(A)** Photomicrographs of C2C12 myoblasts under bright field microscope treated with different concentration of MCE; **(B)** % cell viability of myoblasts treated with MCE (*p <0.05 represents significance w.r.t. control)

Effect of commercial cinnamaldehyde on viability of C2C12 cell lines

Cinnamaldehyde (CNA) and its derivatives have been shown to possess anti-proliferative activity on various cell lines (Lee *et al.*, 1999). Thus, we compared the antiproliferative effect of commercial cinnamaldehyde with that of MCE from the bark of *C. zeylanicum* on C2C12 cell line. MTT test was used to investigate CNA cytotoxicity in C2C12 myoblasts. According to the results, at greater concentrations of 132.16 µg/ml, CNA caused over 60% cell death and dramatically affected the morphology of myoblasts, showing a cytotoxic action of CNA at higher concentrations. Whereas 50% cell death occurs when myoblasts are exposed to 66.08 µg/ml, there is no change in myoblast shape at this concentration, leading us to infer that CNA has an IC_{50} of 66.08 µg/ml. Consistently, another research assessed the cytotoxicity of CNA and found it to be 18 µg/ml, as opposed to 66.08 µg/ml in our study (Nikazmir *et al*., 2014).

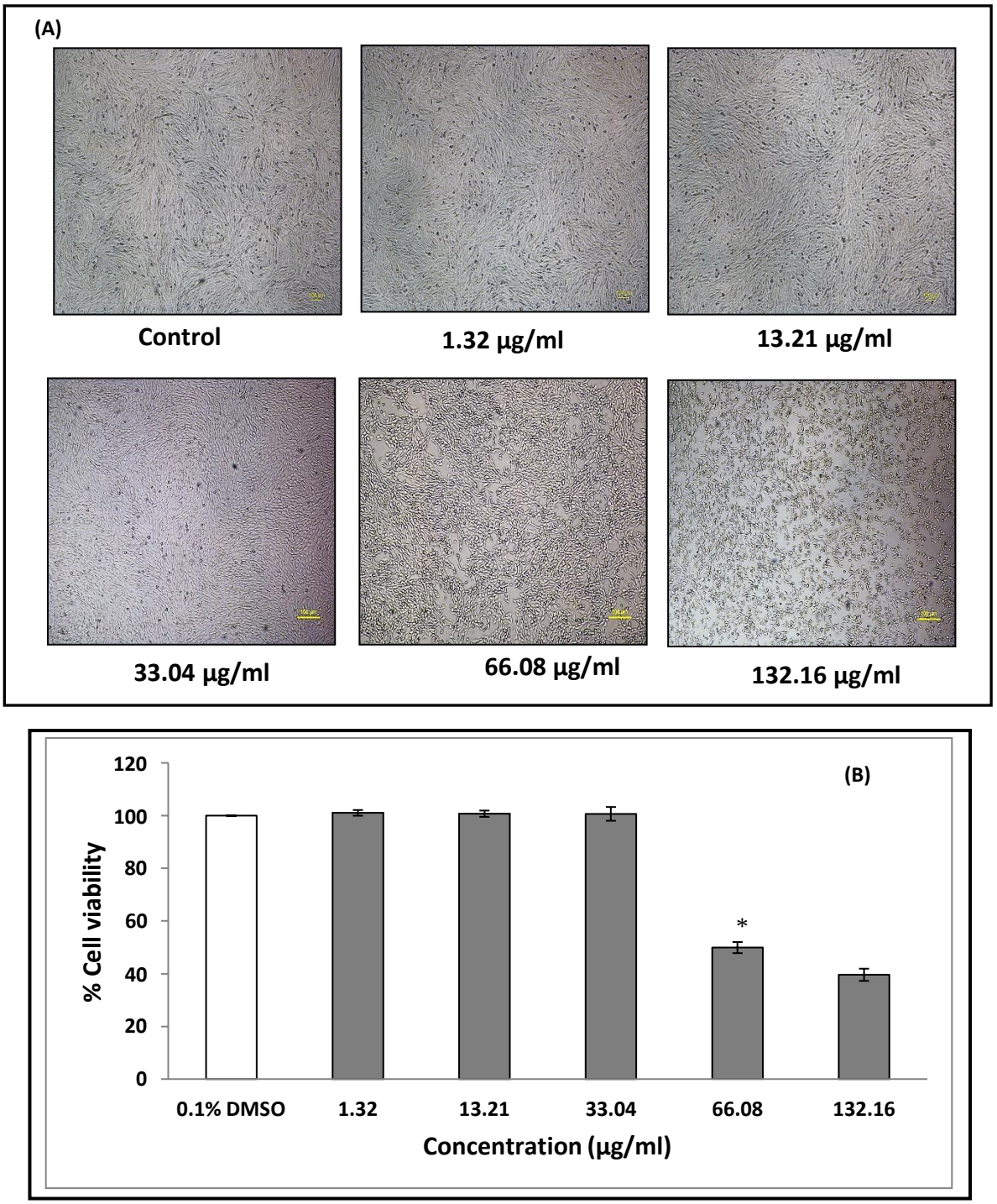

Fig 2: Effect of CNA on C2C12 cells: **(A)** Photomicrographs of C2C12 myoblasts under bright field microscope treated with different concentration of CNA; **(B)** % Cell viability of myoblasts treated with CNA (*p <0.05 represents significance w.r.t. control)

Conclusion

The present study has demonstrated the comparison of cytotoxic effect of methanolic extract of *Cinnamon zeylancium* bark with commercial Trans cinnamaldehyde. It was found that the cinnamaldehye is showing IC_{50} at lower concentration than that of concentrated methanolic *Cinnamon zeylanicum* extract.

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