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Original Research Article

Cytotoxicity of Essential Oil of *Chenopodium ambrosioides* L against Human Breast Cancer MCF-7 Cells

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Abstract

Purpose: *To investigate the cytotoxic activity of the essential oil of Chenopodium ambrosioides L. against human breast cancer MCF-7 cells.*
Methods: *Cytotoxicity was characterized by 50 % inhibition (IC_{50}) of human breast cancer cell lines (MCF-7) using 3-(4,5-dimethylthaizol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Apoptosis was analysed by Hoechst33258 staining and DNA ladder. MCF-7 cellular superoxide dismutase (SOD), catalase (CAT) vitality and malondialdehyde (MDA) content were evaluated.*
Results: *The essential oil was cytotoxic to MCF-7 cell line. A dose- and time-dependent inhibition was observed with IC_{50} values of 18.75, 9.45 and 10.50 μg/ml at 6, 24 and 48 h, respectively. Analyses by Hoechst33258 staining and DNA ladder indicate that the essential oil induced apoptosis. SOD vitality significantly decreased ($p < 0.05$) by 51 % when the concentration of the essential oil increased from 1.25 to 12.5 μg/ml while CAT vitality significantly increased ($p < 0.05$) by 71 % when essential oil concentration was similarly increased. The MDA content of each treatment group, when compare to control, did not show any significant difference ($p < 0.05$).*
Conclusion: *The essential oil of C. ambrosioides was cytotoxic to MCF-7 cell line and induced apoptosis.*

Keywords: *Chenopodium ambrosioides L., Essential oil, Cytotoxicity, Apoptosis, Breast cancer, MCF-7 cells.*

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INTRODUCTION

There are a large number of major secondary metabolites of natural origin with a variety of potential applications [1-4]. Essential oils function basically as primary metabolites. It has many biological activities including allelopathy [2], insecticide resistance [3], antibacterial [4] and antifungal [5]. In the last two decades, there has been increasing interest in the pharmacological activity of essential oils [6]. Several works on essential oils that show their anti-cancer properties have been reported [7,8].

Mexican tea（*Chenopodium ambrosioides* L）is an annual or perennial herb native to tropical America. It is one of the most severely invasive weeds in China. This weed first invaded Danshuei Township of Taipei County in 1864. Currently, it commonly distributes in most areas of China, and produces a great threat to the local ecological system [9]. There is no doubt that utilization of *C. ambrosioides* is an effective approach control the spread of this weed. There have been a lot of studies about its essential oil applications [2-4]. However, there is no report on the inhibition of cancer cell proliferation by the essential oil of *C. ambrosioides*. Only few studies have shown that the hydroalcohol extract of *C. ambrosioides* leaves increased cellular recruitment, phagocytic ability and nitric oxide production [10].

Since *C. ambrosioides* essential oil has been shown to induce cancer cell death, this work sought to investigate the cytotoxic activity of the essential oil on human breast cancer cell lines (MCF-7). The study further examined whether the anti-cancer mechanism of the essential oil involves induction of apoptosis. In addition, the possible mechanism of *C. ambrosioides* essential oil-induced apoptosis was assessed.

EXPERIMENTAL

Plant material and essential oil distillation

Whole plant of *C. ambrosioides* was collected from Chengdu in Sichuan Province (China) in November 2011. A voucher specimen (Collector no. 004) and deposited in the Herbarium of Sichuan Normal University, Chengdu, Sichuan Province, China. The samples were authenticated by Dr. Ma Danwei of Sichuan Normal University.

The essential oil of *C. ambrosioides* was extracted by steam distillation [11]. The extracted oil was dried over anhydrous sodium sulphate and the purified oil was filled in small vials, tightly sealed and stored at 4 ^oC until further analysis.

Cell lines and culture

Human breast cancer line MCF-7 was cultured in RPMI-1640 supplemented with 10% fetal calf serum. Cells were grown and maintained at 37 ^oC in a humidified atmosphere with 5 % CO_2.

Cytotoxicity analysis by the MTT assay

The essential oil was dissolved in DMSO to obtain a concentration of 50 mg/ml which served as stock solution. The stock volume was serially diluted with DMSO (99.5%) to obtain solutions of desired concentrations. *In vitro* cytotoxicity of *C. ambrosioides* essential oil was determined using 3-(4,5-dimethylthaizol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. MCF-7 cells were harvested using trypsin and counted using a haemocytometer. To 1 × 10^4 cells/well, 100 µl of essential oil was seeded in 96 wells. The plates were incubated for 24 h (at 37 ^oC in an atmosphere of 5 % CO_2). Then test concentrations (100 µL/well) were added to the wells containing cells. The plates were incubated for a further 6, 24 and 48 h after addition of test material. The final concentration of DMSO in the culture medium was maintained at 1.0% (v/v) to avoid toxicity of the solvent. After 6, 24 and 48 h, 20 µL of 5 mg/ml MTT in PBS (phosphate buffer solution) was added to each well and then incubated for at 37 ^oC in an incubator. The medium was removed and 150 µL of dimethylsulfoxide (DMSO, Sigma) was added to each well. After shaking for 10 min, the absorbance was measured at 490 nm. Growth inhibition (GI) was calculated formulas in Eq 1.

$$GI\ (\%) = \{(1 - At/Ac)\}100 \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where At is the absorbance of the test sample and Ac the absorbance of the control.

Hoechst DNA staining test

Briefly, MCF-7 cells (5×10^5 cells/ml) were treated with different concentrations of test sample (essential oil) for 6, 24 or 48 h. After the specified time, the processed cells were fixed by 4% polyformaldehyde at 4 ^oC for 10 to 20 min and washed twice with PBS. Then the cells were stained with 5 µg/mL. Hoechst 33258 for 10 min, washed three times with PBS and dried in the dark at room temperature. Lastly, the samples were viewed under a fluorescence microscope (TE2000-U, Nikon, China).

DNA ladder assay

Apoptosis was assessed by electrophoresis of extracted genomic DNA from treated MCF-7 cells as described previously with some modification. Briefly, using varying concentrations (0 - 50 µg/mL) of the test sample (essential oil) MCF-7 cells (1 × 106 cells/mL) were harvested after treatment for 24 h, centrifuged at 2000 rpm for 5 min and washed with PBS. The cells were suspended in 250 µL of lysis buffer (10 mM EDTA, 50 mM Tris-HCl, 0.5 % SDS) at 55 ^oC for 15 min. The lysed cells were incubated with 10 µl of RNase at 55 ^oC for 90 min and digested with 10 µl of proteinase-K (20 mg/ml) at 55 ^oC for 1 h. DNA was extracted twice with isopyknic chloroform: isoamyl alcohol (24:1) for 1 min and centrifuged at 12,000 rpm for 5 min. Finally, DNA was precipitated from the aqueous phase with 0.1 volume of 2 M NaCl and 2.5 volumes of chilled ethanol, and kept at 20 ^oC overnight. The precipitated DNA was centrifuged at 12,000 rpm for 10 min and dissolved in Tris–EDTA buffer (pH 8.0). DNA was subjected to electrophoresis in 1.5 % agarose gel at 50 V for

90 min. The gel was photographed using Gel documentation system (Bio-Rad, Alfred Noble Drive, China).

Determination of antioxidant activity

MCF-7 cells (5×10^5 cells/ml) were treated with varying concentrations (0 - 50 µg/mL) of the test sample (essential oil) for 6, 24 or 48 h. After the specified time, the processed cells were used in the detection of superoxide dismutase (SOD), catalase (CAT) vitality and malondialdehyde (MDA) content. Experimental procedures were conducted according to the kit manufacturer's instructions. SOD, CAT and MDA method were measured by A003-1 kit, A007-1 kit, A003-4 kit, Nanjing Jiancheng Bioengineering Institute, Nanjing, respectively.

Statistical analysis

All the data were expressed as mean ± standard deviation (SD). Statistical analysis was carried out using PASW (version 18.0) software. One-way ANOVA was used for multiple comparisons. A p value of < 0.05 was considered significant.

RESULTS

Cytotoxicity of essential oil of *C. ambrosioides* on MCF-7 cancer cells

The effect of essential oil from *C. ambrosioides* on MCF-7 cells, as assessed by MTT assay, is shown in Fig 1. A dose and time-dependent inhibition was observed with IC_{50} values of 18.75, 9.45 and 10.50 µg/ml at 6, 24 and 48 h, respectively.

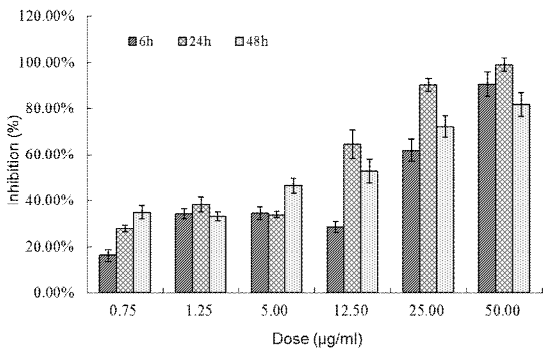

Fig 1: Inhibition of MCF-7 cell growth by *C. ambrosioides* essential oil. Error bars denote standard deviation; n = 3

Effect of essential oil on apoptosis

The results of the use of fluorescence microscopy to visualize MCF-7 cell nucleus after exposure to the essential oil are shown in Fig 2. Compared with control cells (Fig 2-A, D and G), the cells appeared to show condensation and/or fragmentation of nuclei with essential oil concentration of 50 µg/ml at 6 h. However, the phenomenon was observed in cells treated with 12.5 µg/ml essential oil at 24 and 48 h.

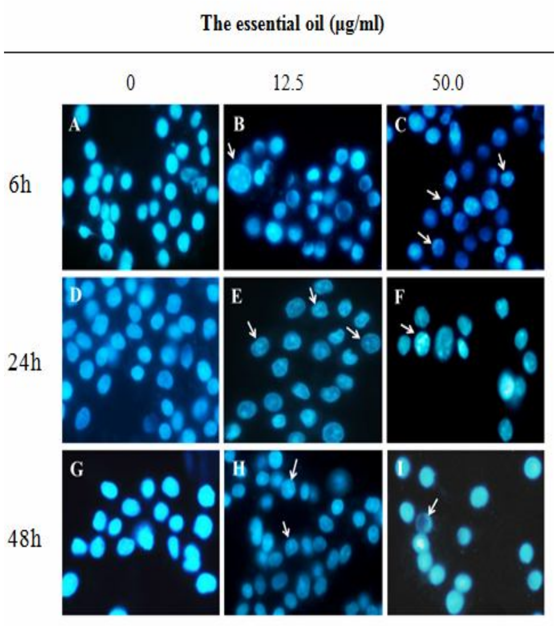

Fig 2: Morphology of MCF-7 cells. ***Note:*** Arrows point to apoptotic cells showing fragmented and/or condensed nuclei

DNA fragmentation

The DNA of the MCF-7 cells treated with the essential oil from *C. ambrosioides* exhibited double-strand damage as observed with agarose gel electrophoresis (Fig. 3).

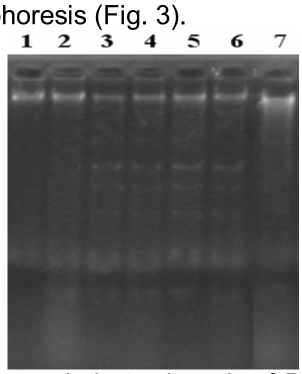

Fig 3: Agarose-gel electrophoresis of DNA extracted from MCF-7 cells untreated and treated with different concentration of *C. ambrosioides* essential oil for 24 h. Lane 1 = control; lane 2 = 0.75 µg/mL oil; lane 3 = 1.25 µg/mL; lane 4 = 5 µg/mL oil; lane 5 = 12.5 µg/mL; lane 6 = 25 µg/mL; lane 7 = 50 µg/mL

Genomic DNA was isolated from the cells exposed to different concentration of the essential oil for 24 h. Efficient induction of apoptosis was observed at 1.25 - 25 µg/mL essential oil treatment by formation of a distinct DNA ladder. The phenomenon of cell death in which DNA is a stage of dispersion appeared after treatment with 50 μg/mL essential oil.

Antioxidant activity

The data for antioxidant activity of the essential oil is shown in Figs 4 - 6 for superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) tests, respectively.

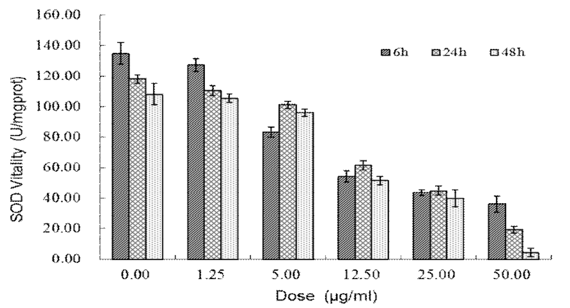

Fig 4: Effect of *C. ambrosioides* essential oil treatment on cellular SOD activity in MCF-7 cells

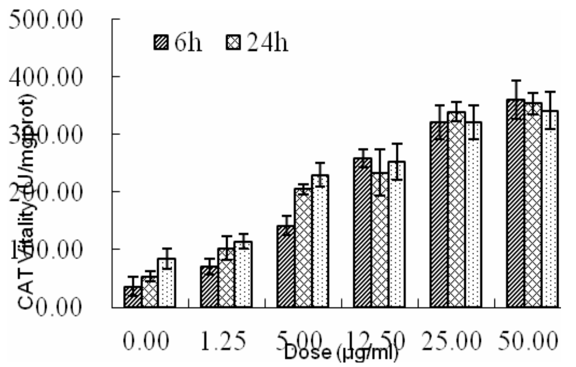

Fig 5: Effect of *C. ambrosioides* essential oil treatment on cellular CAT activity in MCF-7 cells

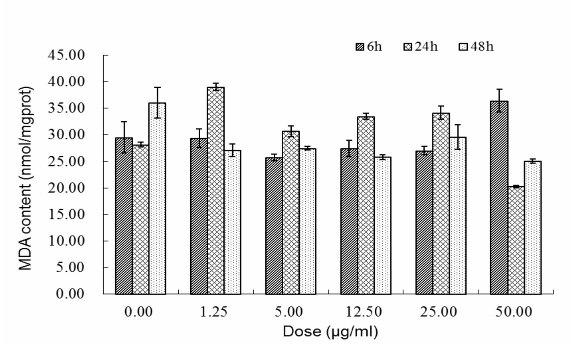

Fig 6: Effects of *C. ambrosioides* essential oil treatment on MDA content in MCF-7 cells

As seen in Fig 4, MCF-7 cellular SOD activity has a negative correlation with essential oil concentration and processing time. *C. ambrosioides* essential oil induced a dose-dependent decrease in cellular SOD activity (51 %) but increase in CAT activity (71 %) as shown in Fig 5. These changes are significant ($p < 0.05$). However, essential oil treatment did not result in significant changes in MDA content between treatment groups ($p < 0.05$, Fig 6).

DISCUSSION

In recent years, considerable attention has been focused on identifying naturally occurring substances capable of inhibiting, retarding, or reversing the process of multistage carcinogenesis [6-8,13]. In the present study, we examined whether or not the essential oil of *C. ambrosioides* has any potential in cancer chemoprevention using MCF-7 human breast cells.

The essential oil inhibited cell proliferation and was cytotoxic in a concentration- and time-dependent manner in MCF-7 cells. Minimum IC_{50} was appeared at 24 h. Compared to another study [12], the IC_{50} of the essential oil was lower, suggesting that the essential oil could exert strong anti-proliferative effect in MCF-7 cells at low dose.

Recently attention has focused on the manipulation of apoptosis processes in the treatment and prevention of cancer [7,13,16]. An essential oil from *Capparis spinosa* L. has been shown to induce apoptosis in human colon carcinoma cell line, HT-29 [6]. The essential oil from *C. ambrosioides* induced morphological alterations typical of apoptotic process, membrane blebbing, chromatin condensation, etc. This observation suggests the presence of increased number of apoptotic cells. DNA ladder is one of the most commonly used techniques for confirmation of apoptosis. The data showed internucleosomal DNA breakdown, leading to DNA fragmentation as expected for apoptosis cells. Thus, *C. ambrosioides* essential oil decreased cell survival by the induction of DNA fragmentation.

In order to confirm the possible mechanism of *C. ambrosioides* essential oil induction MCF-7 cells apoptosis, we investigate the involvement of antioxidant activity in the essential oil induced apoptosis. When the body is under a certain pressure, it will produce excess free radicals [14]. However, excess production of free radical will exacerbate oxidative damage to proteins, lipids and DNA and even lead to cell death [12,15]. The antioxidant data indicate that the essential oil is a weak antioxidant in MCF-7 cells. A previous study also found that the essential oil of A. *tsao-ko* exerted weak antioxidant activity against HepG2 [16]. Thus, the mechanism of induction of MCF-7 cell apoptosis may not be by oxidative damage.

Two major signaling cascades, extrinsic and t intrinsic pathways, lead to apoptosis [12,17]. The extrinsic pathway is triggered by interaction of cell-death factors with their cognate receptors, resulting in activation of caspase-8, which subsequently activates caspase-3. The intrinsic pathway is mainly triggered by non-receptor stimuli that regulate the level of proteins of the Bcl-2 family [13,18]. Increase in the levels of Bax and/or decrease in Bcl-2 lead to loss of MMP and release of pro-apoptotic factors such as cytochrome c from mitochondria to the cytosol, resulting in activation of caspase-9 and subsequent activation of caspase-3 [13,17]. Therefore, one pathway can be amplified by crosstalk with another pathway. Therefore, the mechanism which the essential oil of *C. ambrosioides* reduced the MCF-7 cells apoptosis needs further investigation.

CONCLUSION

This work demonstrates that the essential oil of *C. ambrosioides* is cytotoxic to MCF-7 cells by a mechanism related to apoptosis, and that it is a weak antioxidant. Further studies are needed to determine the effective constituents of the oil and to elucidate their roles in achieving cytotoxicity. The mechanism by which the essential oil induces cell apoptosis also requires further investigation.

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