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Induction of apoptosis in melanoma A375 cells by a chloroform fraction of *Centratherum anthelminticum* (L.) seeds involves NF-kappaB, p53 and Bcl-2-controlled mitochondrial signaling pathways

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Abstract

Background

Centratherum anthelminticum (L.) Kuntze (scientific synonyms: *Vernonia anthelmintica*; *black cummin*) is one of the ingredients of an Ayurvedic preparation, called "Kayakalp", commonly applied to treat skin disorders in India and Southeast Asia. Despite its well known anti-inflammatory property on skin diseases, the anti-cancer effect of *C. anthelminticum* seeds on skin cancer is less documented. The present study aims to investigate the anti-cancer effect of *Centratherum anthelminticum* (L.) seeds chloroform fraction (CACF) on human melanoma cells and to elucidate the molecular mechanism involved.

Methods

A chloroform fraction was extracted from *C. anthelminticum* (CACF). Bioactive compounds of the CACF were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Human melanoma cell line A375 was treated with CACF *in vitro*. Effects of CACF on growth inhibition, morphology, stress and survival of the cell were examined with MTT, high content screening (HSC) array scan and flow cytometry analyses. Involvement of intrinsic or extrinsic pathways in the CACF-induced A375 cell death mechanism was examined using a caspase luminescence assay. The results were further verified with different caspase inhibitors. In addition, Western blot analysis was performed to elucidate the changes in apoptosis-associated molecules. Finally, the effect of CACF on the NF-κB nuclear translocation ability was assayed.

Results

The MTT assay showed that CACF dose-dependently inhibited cell growth of A375, while exerted less cytotoxic effect on normal primary epithelial melanocytes. We demonstrated that CACF induced cell growth inhibition through apoptosis, as evidenced by cell shrinkage, increased annexin V staining and formation of membrane blebs. CACF treatment also resulted in higher reactive oxygen species (ROS) production and lower Bcl-2 expression, leading to decrease mitochondrial membrane potential (MMP). Disruption of the MMP facilitated the release of mitochondrial cytochrome c, which activates caspase-9 and downstream caspase-3/7, resulting in DNA fragmentation and up-regulation of p53 in melanoma cells. Moreover, CACF prevented TNF-α-induced NF-κB nuclear translocation, which further committed A375 cells toward apoptosis.

Conclusions

Together, our findings suggest CACF as a potential therapeutic agent against human melanoma malignancy.

Keywords: *Centratherum anthelminticum*; Melanoma; Caspase cascade; Apoptosis; Bcl-2; p53; NF-κB

Background

Melanoma is a skin cancer that arises from the malignant transformation of melanocytes. Epidemiological studies showed that the incidence of melanoma is increasing at a rate faster than that of any other cancers worldwide [1-3]. Moreover, although early stage melanoma is confined to epidermis and is curable, metastasized melanoma has an unfavourable prognosis, where the overall survival for patients with metastatic melanoma ranges from 4.7 to 11 months, with a median survival of 8.5 months [4]. This poor prognosis is due to the lack of effective treatment options [5].

Melanoma is often characterized by resistance to cytotoxic agents which contributes to the high morbidity and mortality rates in patients. Therefore it is important to look for new sources of anti-cancer agents that exert cytotoxicity activity against melanoma cells. Plant extracts have been used as complementary medicine for many years. Various phytoconstituents that possess multiple biological and synergistical effects in the plant extracts can function to treat different ailments or enhance the effect of drugs [6-9]. Certain natural products have been applied for cancer chemoprevention to inhibit or revert carcinogenesis and to suppress the malignancy of cancer [10].

Centratherum anthelminticum (L.) Kuntze seeds (scientific synonyms: *Vernonia anthelmintica*) is commonly known as black cummin and widely used as curry spice mixtures. Studies have shown various pharmacological properties exhibited in the seeds of *C. anthelminticum*, such as anti-viral, anti-filarial, anti-microbial, anti-