Research Article

Apoptotic induction of *Cardiospermum halicacabum* on oral cancer cell lines

V. J. Oviya, T. Lakshmi*

ABSTRACT

Introduction: Cancer has always been considered as a deadly disease. Taken as a whole, about half of the patients suffering from invasive cancer receiving treatment, die from its treatment or cancer itself. A requirement for a finding of new ways of treating cancer would be essential to be able to save the lives of those with cancer rather than just extending their lives by a few weeks. Hence, in this study, apoptotic induction potentials of *Cardiospermum halicacabum* extract along oral cancer cell line were studied. **Materials and Methods:** A fresh extract of *C. halicacabum* was used. Apoptotic induction potentials of the extract were studied by analyzing the caspase activities (caspase-3 and caspase-9) using chromogenic assays. **Results:** In this study, apoptotic induction potential of various concentrations of *C. halicacabum* extract (50, 100, and 150 µg) on KB cells was studied by monitoring the activation of caspase-3 and caspase-9. Activity of caspase-3 was more compared to caspase-9. **Conclusion:** The mechanism that can activate caspases may, therefore, represent a possibly feasible approach for effective tumor treatment which has several advantages over both conventional therapies and the more current "designer" approaches. The secondary metabolites from herbs are always promising with antioxidant and anticancer activity. The ability of apoptotic induction by *C. halicacabum* extract can beO utilized in anticancer formulation.

KEY WORDS: Anticancer, Antioxidant, Caspase-3, Conventional, Oral cancer

INTRODUCTION

Oral cancer is one of the 10 most frequently occurring cancers worldwide, and its incidence in Europe and the United States ranges from 2% to 6% among all cancer patients.[1,2] Treatment of oral cancer has primarily relied on classical modalities such as surgery, radiation, and chemotherapy or a combination of these methods. Many of the currently used antimitotic drugs were developed on the premise that cancer is fundamentally a disease of enhanced or sustained cell proliferation.[3] However, efforts to eradicate these disseminated neoplastic cells often have resulted in adverse systemic and cytotoxic effects and development of resistance to therapy. In addition, drug-induced cell damage does not inevitably lead to tumor cell death, in part due to evasion of apoptosis by cancer cells.[4]

Apoptotic cells are characterized by a number of distinct morphological and biochemical changes such as cell shrinkage, cytoplasmic bleb formation, nuclear condensation, and DNA fragmentation.[5] A great number of apoptosis-regulating genes have recently been discovered which includes bc1-2, c-myc, and p53. Apoptosis acts as an important factor in regulation of tumor proliferation and also tumor response to various forms of cancer therapy which includes chemotherapy and radiotherapy. This has been suggested by recent experimental evidence.[6]

Surgical treatment for oral cancer can cause functional and esthetic impairment, leading to withdrawal and social isolation complications of radiotherapy can impair wound healing and further complicate surgical salvage after a failed procedure.[7] Hence, alternative and less toxic chemical treatments for oral cancer are required. Therefore, in recognition of nature's potential, several plants screenings have performed since 1960 to replace drugs that have side effects.[8]

There is a necessity for research to the search of new compounds with cytotoxic activity, as the treatment of cancer. The available anticancer drug is often unsatisfactory due to the problem, which causes cytotoxicity to the normal cells along with cancer

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cells. Plants are considered as the valuable sources of bioactive compounds with antioxidant activity, which produce certain substances that have effects on living animal cells.[9]

Cardiospermum halicacabum Linn. is commonly referred as balloon vine or love in a puff and it belongs to the family Sapindaceae. Cardiospermum is derived from two Latin words including cardio, meaning heart while sperma, meaning seed denoting the white heart-shaped pattern on the seed. Helicacabum is a plant with Brown inflated fruits and derived from the Latin word helicacabus. It is an annual or occasionally a perennial climber commonly distributed in tropical and subtropical Africa and Asia. It has been examined for antidiarrheal as well as homeopathic medicinal properties. *C. halicacabum* has been used in the treatment of rheumatism, nervous diseases, stiffness of the limbs, and snakebite.[10]

Hence, in this study, the apoptotic induction of *C. halicacabum* on oral cancer cell lines was analyzed by its caspases activity using chromogenic assay.

MATERIALS AND METHODS

Preparation of *C. halicacabum* Extract

The stumps of mature *C. halicacabum* plants were stripped of leaves and then pared to remove the roots and the epidermal layer. The cleaned stumps were then quartered and fed into a three roll experimental sugar mill press. The pressed residue was again rerun through the mill for an extra 3 times. Water was added to the pulp to increase the efficiency of the extraction process.

Maintenance of KB Cell Lines

Cell culture flasks were selected by the method of confluency. They were observed under inverted microscope. To maintain the cell line, the technique of subculturing was performed, in which cells were extracted from the existing medium and placed into a fresh new medium to facilitate growth. For cell maintenance, enzymatic methods using Trypsin Phosphate Versene Glucose were prevalently used. Growth medium is then extracted completely from the flasks and the cell was further subjected to incubation at 37°C after the addition of the enzyme. This, initially, detaches the cells from the surface.

The cells were then suspended in 5 ml of the medium. This suspension was repeated a few times to break the cell clumps. The date of seeding, cell line, and the passage number were then marked on the bottom of the T-flask. The cell suspension in 5 ml of medium was then transferred into a fresh new T-flask.

Determination of Caspase Activity

Caspase activities were determined by chromogenic assays using caspase-3 and caspase-9 activation kits according to the manufacturer's protocol (Calbiochem, Merck). After treating with designated compounds, the cells were lysed using lysis buffer (50 mM HEPES, 100 mM NaCl, 0.1% CHAPS, 1 mM DTT, and 100 mM EDTA). Lysates were centrifuged at 10,000 rpm for 1 min. The supplements (cytosolic extract) were collected and protein concentration was determined by the Bradford assay using bovine serum albumin as a standard. 100–200 µg protein (cellular extracts) was diluted in 50 µL cell lysis buffer for each assay. Cellular extracts were then incubated in 96-well microtiter plates with 5 µL of the 4 mM p-nitroanilide (pNA) substrates, DEVD-pNA (caspase-3 activity) for 2 h at 37°C. Caspase activity was measured by cleavage of the above sub-substrates to free pNA. Free pNA (cleaved substrates) was measured by absorbance at 405 nm in a microtiter plate reader. Relative caspase-3 and 9 activities were calculated as a ratio of the absorbance of treated cells to untreated cells.

RESULTS

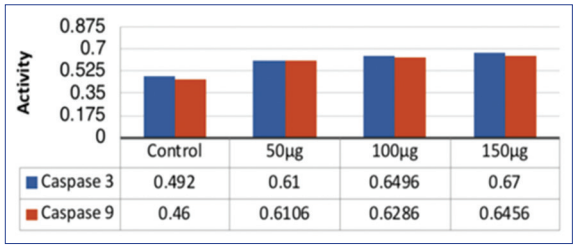
In this study, apoptotic induction potential of various concentrations of *C. halicacabum* extract (50, 100, and 150 µg) on KB cells was studied by monitoring the activation of caspase-3 and caspase-9.

From the results, it is clear that the activity of caspase-3 showed 0.492 which got increased when the concentration increased by 50 µg with value 0.61, with 100 µg, it showed 0.64 and with 150 µg, the activity got increased to 0.67 [Figure 1].

From the graph, it is evident that caspase-9 also increased its activity with increase in concentration. Again still control, it showed 0.46, while with 50 µg, its activity increased to 0.61 µg, at 100 µg, it got increased to 0.62, and with 150 µg, it got increased to 0.64 µg/ml.

DISCUSSION

Herbal medicines play an important role in health-care programs in the developing countries. A remarkably broad definition of medicinal plants to the potential sources of medicinal substances was incorporated by ancient Indian literature. Diseases that remain most



	Control	50µg	100µg	150µg
Caspase 3	0.492	0.61	0.6496	0.67
Caspase 9	0.46	0.6106	0.6286	0.6456

Figure 1: Bar graph showing the activity of different caspases by *C. helicacabum*

challenging for today's health-care system tend to be more complex than could be treated by current combination therapies.

Apoptosis is considered a vital component of various processes which include turnover of a normal cell, proper functioning and development of the immune system, hormone-dependent atrophy of cells, embryonic development, and chemical-induced cell death.[11]

The conventional anticancer drugs which are being used, act on both normal cells and tumor cells, causing brutal side effects and tumor resistance. Anticancer activity by apoptotic induction by herbs does not show any side effects.[12]

Caspases, also known as cysteinyl aspartate-specific proteases, are a family of important signaling molecules. The activation of caspases also acts as a marker for damage in cellular level of diseases such as stroke and myocardial infarction. These caspases are responsible for the programmed cell death or apoptosis. It begins by the activation of intrinsic and extrinsic pathways. Once activated these destructive proteases proceed to systematically deconstruct the cell to ensure its effective removal without damage to surrounding cells and tissues.[13]

Even though caspases playing the precise role in the initiation and progression of apoptosis are not known, their role as an indicator alone and as a potential leverage point for research of drug makes them largely researched molecules.[14]

So far, caspase-3 has clearly emerged as the single most important caspase during the execution phase of apoptosis. Using cells derived from caspase-3-deficient mice, Zheng *et al*. have shown that dying caspase-3 cells undergo an aberrant form of apoptosis, exhibiting drastically delayed cellular changes such as cytoplasmic bleb formation and nuclear and DNA fragmentation.[15]

In this study, caspase-3 had higher activity compared to caspase-9 with increased concentration of extract. Similar study was done by Nadhirah *et al*. on apoptotic induction potential of various concentrations of pineapple extract and analyzed the increased activity of caspase-3 compared to caspase-9.

Annadurai *et al*. studied and verified the traditional use of *C. halicacabum* L. for human ailments and partly explained its use in herbal medicine as a rich source of phytochemicals with the tannin, flavonoid, terpenoid, cardiac glycosides, alkaloids, and anthrax quinones.[16]

CONCLUSION

The plant extractive studies could be an answer to the people seeking for better therapeutic agents from natural sources. It is considered to be more efficient with little or no side effects comparing the effects of commonly used synthetic chemotherapeutic agents. This study demonstrates that plant extract of *C. halicacabum* inhibits cell growth and induces apoptosis in human oral cancer cell lines. The results implied that this extract could be a promising traditional herbal medicine for potential use in oral cancer therapy which can lead to a promising strategy for oral cancer therapy.

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