PURIFIED COMPOUND 4,8-DIHYDROXYBENZO(DE)CHROMEN-2(4H)-ONE FROM *CAESALPINIA CORIARIA* (JACQ) WILLD - INDUCED CASPASES ACTIVITY RELATED TO APOPTOSIS IN A-549 CELLS

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ABSTRACT

In order to assess the mechanism of bioactive compound 4,8-dihydroxybenzo(de)chromen-2(4H)-one induced apoptosis, we have evaluated the caspase activity. The activation of the caspases (cysteine aspartic-specific proteases) is the classical investigation for the mechanism of apoptosis. Caspases are classified into upstream regulatory caspases and downstream effector caspases. The upstream caspases such as caspase-8 (death receptor pathway) and caspase-9 (mitochondria pathway), with their N-terminal prodomain interact with and activate the proapoptotic proteins, including other caspases. In the present study *C.coriaria* induced the apoptotic signaling by the bioactive compound was mainly related to the mitochondrial pathway. In this study the expressions of the caspase-3 and caspase-9 after treatment with the bioactive compound is dose dependent. The activity of enzyme gradually increases from 113.09 to 142.619 for caspase-3 and 106.15 to 134.587 for caspase 9. Caspase-9 and caspase-3 could be activated during apoptosis was due to the purified compound 4, 8-dihydroxybenzo (de)chromen-2(4H)-one. These results showed that the bioactive compound induced apoptosis in A-549 cells, through caspase-9 and caspase-3 activation. In conclusion the anticancer activity of the purified compound of *C.coriaria* induces apoptosis in A-549 cells, accompanying by the enhanced activity of caspase-9 and caspase-3.

Keywords: *Caesalpinia coriaria,* 4, 8-dihydroxybenzo (de)chromen-2(4H)-one, caspase-3 and caspase-9, A-549 cells

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INTRODUCTION

Medicinal herbs play a major role in anticancer remedies. Herbal medicines also play a major role in the prevention and treatment of cancer. Based on the knowledge of molecular techniques and constructive in isolation and structure elucidation techniques, a huge number of anticancer herbs were identified. Herbal medicine will boost immune cells of the body against cancer. By understanding the synergistic action of the pytochemicals of anticancer herbs, it can be designed to affect the cancerous cells without destructing the normal cells of the body (Larkin, 1983, Saxe, 1987). A survey of plants, microorganism and marine animals for antitumor activity began in the later 1950s by United States National Cancer Institute (NCI).

APOPTOSIS

Apoptosis is a process for the selective removal of cells, which is responsible in a number of biological events. The Bcl-2 family is the best protein involved in the regulation of apoptotic cell death using anti-apoptotic and pro-apoptotic members. The anti-apoptotic members of this family are Bcl-2 and Bcl-XL, prevent the apoptosis either by the activations of cysteine proteases called caspases or by regulating the release of mitochondrial factors namely cytochrome c and AIF (apoptosis-inducing factor) in the cytoplasm. The entry of cytochrome c and AIF directly stimulate the caspases to cause apoptotic changes. The pro-apoptotic members of this family such as Bax and Bak stimulate the release of caspases and for inducing the cell death. Bcl-2 family of proteins play a critical life-death (Tsujimoto, 2013). Caspase-9 also termed as Mch6, Apaf-3 or ICE-LAP-6 is present in cells as an inactive 46 kDa proenzyme called pro-caspase-9. Pro-caspase -9 is cleaved to 35 and 10kDa subunits by Cytochrome C during apoptosis. The downstream substrates for caspase-9 include caspase-3 and -7. Caspase 3 is a protein that interacts with caspase 8 and caspase 9. It is encoded by the *CASP*3. Caspase-3 shares many characteristics common to all known caspases. The catalytic site of caspase -3 involves the sulfhydryl group of Cys-285 and the imidazole ring of His-237. Caspase -3 has the specificity in cleavage of peptide sequence DEVDG (Asp-Glu-Val-Asp-Gly) with cleavage site on the carboxyl side of the second asp amino acid (Stennicke, 2000, Lavrik, 2005, Porter, 1999) **.** Drug discovery is a complex undertaking facing many challenges (Schmid, 2005), not the least of which is a high attrition rate as many promising candidates prove ineffective or toxic in the clinic

owing to a poor understanding of the diseases, and thus the biological systems, they target. Therefore, it is broadly agreed that to increase the productivity of drug discovery one needs a far deeper understanding of the molecular mechanisms of diseases, taking into account the full biological context of the drug target and moving beyond individual genes and proteins (Fishman, 2005, Butcher, 2004, Apic, 2005).

MATERIALS AND METHODS

PLANT DESCRIPTION

The samples (pods) of *Caesalpinia coriaria* were collected from Captain Srinivasa Murti Drug and Ayurveda Research Institute, Chennai and was identified from Dr. Jayaraman. The samples were allowed to dry under the shade completely. The dried pods were ground into powder, which was used for further extraction.

SAMPLE PREPARATION OF *C.CORIARIA* EXTRACT

Caesalpinia coriaria ethanolic pod extract of different fractions I, II, III, IV, V 20 mg/ 10 ml PBS was dissolved. The extracts were vortexed for 30 minutes. 200 µl of DMSO (sigma, St. Louis, USA) was added to all the fractions. For all the experiments these prepared extracts were used.

CELL LINES AND CULTURE MEDIUM

SiHa (Cervical Cancer), MCF-7(Breast Cancer), A549 (Lung Cancer) and Vero (Normal Cell Line) were purchased from NCCS, Pune and cultures in Dulbecco's modified Eagle medium (Sigma, St. Louis, USA) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin (Sigma, St. Louis, USA). The cells were then incubated in a humidified atmosphere at 5% CO_2 at 37 ^{o}C. All the cell lines used in the study were of passage number between 3 to 7.

NMR STRUCTURAL ELUCIDATION

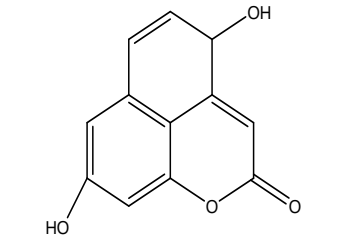
NMR was used as analytical tool for predicting the structure of the molecule based on the different environments of hydrogen atom by measuring the magnetic moments of hydrogen atom. Purified samples were subjected to NMR studies. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker FT NMR spectrometer and chemical shifts were recorded in ppm in Indian Institute of Technology, Chennai.

DETERMINATION OF CASPASE ACTIVITY

Caspases activities were determined by colorimetric assays using caspase-3 and caspase-9 activation kits according to the manufacturer's protocol (Calbiochem merck). After treated with designated concentrations of compound (25, 50 and 75µg, cell lysates were prepared by incubating 2 X 10^6 cells/ml in cell lysis buffer (50mM HEPES, 100mM NaCl, 0.1% CHAPS, 1mM DTT, 100mM EDTA) for 10 min on ice. Lysates were centrifuged at 10,000 X g for 1 min. The supernatants (cytosolic extract) were collected and protein concentration was determined by the Lowry's method using BSA standard. 150 µg proteins (cellular extracts) were diluted in 50 µl cell lysis buffer for each assay. Cellular extracts were then incubated in 96-well microtitre plates with 5 µl of the 4mM p-nitroanilide (pNA) substrates, DEVD--pNA (caspase-3 activity) and Ac-LEHD - pNA (caspase -9 activity) for 2 hr at 37 ◦C. Caspase activities were measured by cleavage of the above substrates to free pNA. Free pNA (cleaved substrates) was measured by absorbance at 405nm in a microtitre plate reader. Relative caspase-3 activity was calculated by the ratio of the absorbance of treated cells to untreated cells.

RESULTS AND DISCUSSION

Characterization of *C.coriaria* compound by ^{13}C NMR Spectrum

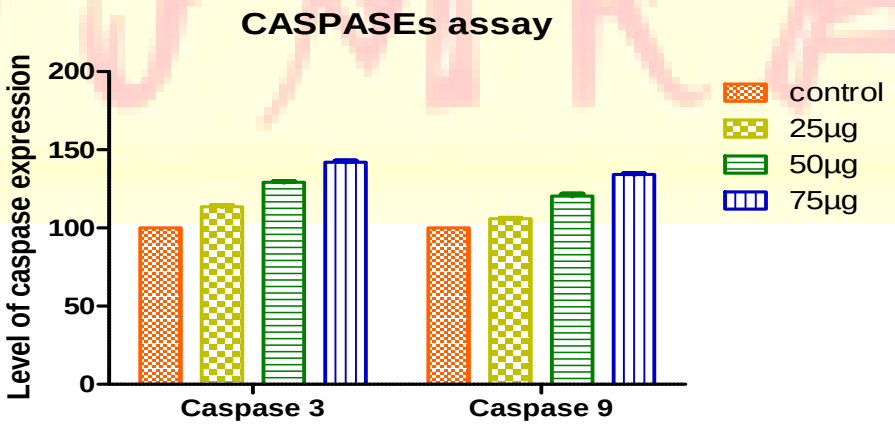


4,8-dihydroxybenzo[*de*]chromen-2(4*H*)-one

Fig 1 – Structure of the isolated compound from *C.coriaria*

PURIFIED BIOACTIVE COMPOUND FROM *C.CORIARIA* INDUCED CASPASE PROTEIN EXPRESSION RELATED TO APOPTOSIS IN A-549 CELLS

In order to assess the mechanism of bioactive compound induced apoptosis, the caspase activity was evaluated by colorimetric assay. Caspases are cytosolic proteins that present normally as zymogen form with high molecular weight (46,320 kDa), (Srivastava, 2011). Caspases are cleaved proteolytically into lower molecular weights (20-23 kDa) during which the cells undergo apoptosis (Solary, 2001). In this study the expressions of the caspase-3 and caspase-9 after treatment with the bioactive compound was dose dependent (Graph 1) The activity of enzyme gradually increased from 113.09 to 142.619 for caspase-3 and 106.15 to 134.587 for caspase 9 respectively.



Graph 1 The levels of expression of Caspase 3 and 9 in A-549 treated cells.

CONCLUSION

The contribution of medicinal plants in the traditional medicinal system and in thediscovery of modern medicine have been analyzed through the literature from 1975-2012. From the systematic analysis of the literature it was apparent that the mode of action and the compound which is responsible for anticancer activity of *C.coriara* has not been explored.

The phytochemicals were screened, isolated and characterized through ^{1}H, ^{13}C NMR and the structure was elucidated as 4,8-dihydroxybenzo(de)chromen-2(4H). In conclusion the anticancer activity of the purified compound of *C.coriaria* induces apoptosis in A-549 cells, accompanying by the enhanced activity of caspase-9 and caspase-3.

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