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Ethanolic extract of the Goldenseal, *Hydrastis canadensis*, has demonstrable chemopreventive effects on HeLa cells *in vitro*: Drug–DNA interaction with calf thymus DNA as target

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ABSTRACT

This study tested chemotherapeutic potential of *Hydrastis canadensis* (HC) extract in HeLa cells *in vitro*, with emphasis on its drug–DNA interaction and apoptosis induction ability. Nuclear uptake of HC by DAPI, Ao/Eb staining and internucleosomal DNA damage by comet assay was studied through fluorescence microscopy. Possible changes in MMP and apoptotic signalling events were critically analyzed. Cell cycle progression studied through FACS and fragmented DNA through "TUNEL" assay were critically analyzed. RT-PCR studies were conducted for analyzing Cyt-C and Bax translocation in mitochondrial and cytosolic extracts, and Caspase 3 in whole cell lysate. Role of p53-mediated regulation of NF-κβ and TNF-α was elucidated by Western blot analysis. Data of CD and Tm profile of CT-DNA were analyzed. Overall results indicated anti-cancer potential of HC through its ability to induce apoptosis, and interaction with CT-DNA that changed structural conformation of DNA, proving HC to be a promising candidate for chemoprevention.

Abbreviations: HC, *Hydrastis canadensis*; DAPI, 40, 60-diamidino-2 phenyl indole; Ao/Eb, acridine orange/ethidium bromide; MMP, mitochondrial membrane potential; FACS, fluorescence activated cell sorting; TUNEL, terminal deoxynucleotidyltransferase dUTP nick end labelling; RT-PCR, reverse transcription polymerase chain reaction; Cyt-C, cytochrome C; NF-κβ, nuclear factor-κβ; TNF-αtumour, necrosis factor-α; CD, circular dichroism; Tm, melting temperature; CT-DNA, calf thymus DNA; CAM, complementary and alternative medicine; DMEM, Dulbecco's modified Eagle medium; FBS, fetal bovine serum; EDTA, ethylene diamine tetra-acetic acid; PSA, penicillin–streptomycin–amphotericine; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide; PI, propidium iodide; BrdU, bromodeoxyuridine; Br-dUTP, 5-bromo-2′-deoxyuridine-5′-triphosphate; dNTPs, deoxynucleoside triphosphates; TdT, terminal deoxynucleotidyltransferase; BCIP-NBT, 5-bromo-4-chloro-3-indolyl phosphate-nitro blue tetrazolium; NCCS, National Centre for Cell Science; LDH, lactate dehydrogenase; PBS, phosphate buffer saline.

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1. Introduction

Chemotherapeutic agents are important in treating cancer, but due to their rather non-selective nature and dose-limiting toxicity, their use is often precluded or restricted, necessitating search for newer drugs having greater potential and suitability of use. The plant kingdom is a rich source of various drugs, and it needs careful studies to find out the right kind of drug having a strong chemotherapeutic effect. Therefore, pharmacological studies on drugs showing potential of their use in oncology are always welcome. In recent years, alternative approach made in the regimen of CAM including homeopathy has been yielding good results (Bensoussan et al., 2006), and therefore becoming a popular mode of therapy, particularly in oncology, although the orthodox approaches for cancer therapy including surgery, radiotherapy and adjuvant chemo- or hormone-therapies cannot be totally ruled out in specific cases. However, due to increased rate of developing resistance against the standard chemotherapy practices, search is on for finding out more suitable alternative remedies that can effectively treat cancer and overcome the problem of drug resistance. For this reason, the possibility of use of herbal medicine, folk medicine, Chinese medicines or even dietary supplements/food with anti-oxidant, anti-inflammatory and anti-allergic activities are being seriously explored using both *in vivo* and *in vitro* models (Adetutu et al., 2012), so that adequate evidence-based research findings can be provided to validate and justify their use in various traditional medicine systems.

Products obtained from dried roots of HC, commonly known as Goldenseal, are generally used in various systems of traditional medicine including homeopathy for the treatment of various health disorders, such as, gonorrhoea, infectious diarrhoea, eye infections, vaginitis etc. In homeopathy, the ethanolic crude extract of HC, known as the mother tincture of HC, is also occasionally used to treat cancer, but it lacks a systematic experimental validation. HC has been reported to contain major bioactive compounds like isoquinoline alkaloids berberine, hydrastine, palmatine and canadine (Weber et al., 2003). Although Karmakar et al. (2010) conducted *in vivo* studies in mice model and demonstrated anti-carcinogenic potential of HC on the basis of some enzymatic studies, no studies had so far been conducted on the chemotherapeutic potential of this drug on HeLa cells *in vitro*, and particularly on its possible DNA binding abilities leading to apoptosis.

Therefore, the hypotheses to be tested in the present study were: (a) if HC has any cytotoxic effect on HeLa cells; (b) if it has, to track down the possible signalling events leading to apoptosis; (c) to examine if HC has DNA-binding ability sought for anti-cancer drugs by using CT-DNA as target and analysing the CD spectra and Tm profile of CT-DNA, and to ascertain (d) if HC can bring about any conformational change in the structure of DNA; and (e) if so, to elucidate the possible relationship between its DNA binding behaviour and signalling cascades.

2. Material and methods

2.1. Chemicals and reagents

DMEM, FBS, trypsin and EDTA were purchased from Gibco BRL (Carlsbad, CA, USA). PSA antibiotic from Himedia (Mumbai, India), and tissue culture plastic wares were obtained from Tarsons (Kolkata, India). DAPI, MTT, PI, mouse secondary-FITC and all other chemicals used were purchased from Sigma Chemical Co. (St Louis, USA). Monoclonal antibodies against NF-κβ and P53, polyclonal antibody against TNF-α and secondary antibodies (mouse, rabbit) were procured from Santa Cruz Biotechnology (Santa Cruz, CA, USA). BrdU-mouse monoclonal antibody was procured from Cell Signaling Technology (USA) and Br-dUTP from Boehringer Manheim, Germany, M-MuLV reverse transcriptase, Taq DNA polymerase, dNTPs, oligonucleotide primers, other RT-PCR reagents, TdT and BCIP-NBT were procured from Chromous Biotech (Bangalore, India). All other chemicals used were procured from Sigma Chemical Co., if not mentioned otherwise.

2.2. Plant extract of HC and dose selection

Ethanolic root extract of HC, used as a homoeopathic mother tincture, was procured from BOIRON Laboratories (Lyon, France). The extract was lyophilized to evaporate the solvent, keeping the constituents of the extract unaltered to obtain its dry weight. It was found that 1 ml of the plant extract is equivalent to 19 mg of its dry weight. Cells were treated at the final doses of 95, 190, 285, 380, 475 and 570 μg/ml (w/v), respectively.

2.3. Cell culture and treatment

HeLa cells were obtained from NCCS, Pune, India. Cells were routinely maintained in DMEM supplemented with 10% FBS and 1% antibiotic at 37 °C in a humidified incubator containing 5% CO_2. For HC treatment, appropriate volumes of HC were added to the cell cultures to achieve the indicated concentrations mentioned above and then incubated for the stipulated amount of time. Cells without any treatment were considered as control. To check the alcohol effect (vehicle/solvent) on cells, solvent control was also placed.

2.4. Determination of cell viability (MTT assay)

Cytotoxic effect of HC on HeLa cell was determined by MTT assay. Briefly, cells were seeded in 96-well plates at a density of 5×10^3 cells/well and treated with HC in various concentrations (95, 190, 285, 380, 470 and 570 μg/ml) at 37 °C for 24 h. After the exposure period, 10 μl of MTT (5 mg/ml) was added into each well and incubated at 37 °C for 4 h. The purple-coloured precipitate of formazan, proportional to viable cells was dissolved in 100 μl of acidic isopropanol. The colour absorbance of each well was recorded at 595 nm with a (Multiscan EX, Thermo Electron Corporation, USA) microplate reader (Tang et al., 2009).