

ANTIPROLIFERATIVE ACTIVITY OF COCCINIA GRANDIS AGAINST CANCER CELL LINES

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Introduction:

Cancer is the most devastating diseases, having significant social and economic impacts on the health care system and second leading cause of death in both men and women worldwide[1,2,3,]. Cancer can be characterized by the failure in the regulation of tissue growth results in the uncontrolled multiplication of the normal cells to form tumors which in further invades into nearby parts of the body [4]. It is estimated that every year, 10 million people are diagnosed with cancer, and of these, 6 million was die of this disease [5].

Treatment to cancer has being researched on a large scale however, resistance to anticancer drugs has also been observed. Therefore, natural products from plants play a dominant role in the discovery of such new drugs. It has been estimated that about 60% of approved drugs were of natural origin [6]. Over 50% of drugs used in clinical trials for anticancer activity were isolated from natural sources or are related to them [7]. Hence, the search for natural products to be used in cancer therapy represents an area of great interest in which the plant kingdom has been the most important source, providing many anti-tumor agents with novel structures and unique mechanism of action [8].

Many substances derived from dietary or medicinal plant known to be effective and versatile chemo preventive and antitumor agents in a number of experimental models of carcinogenesis. The cytotoxicity of plant material is considered to be due to the presence of antitumor compounds [9].

Coccinia grandis is one of the many vegetables that reduce the risk of cancer. The anticancer activity of the Coccinia grandis may be due it's the antioxidant nature. Nitric oxide is a free radical which acting an important role in the pathogenesis of pain and inflammation. The antioxidant principle of Coccinia grandis decreases the nitrite generated by decomposition. Coccinia grandis significantly reduced viable cell count and increased non-viable cell count suggesting comparable anticancer property with that of the reference drug (vinblastine) [10].

Materials and methods:

Collection and culturing of cell lines:

The cytotoxic HEPG-2 cell lines were collected from the National Centre for Cell Sciences (NCCS), Pune. The cells were maintained in Minimal Essential Media (MEM; HiMedia) supplemented with 10% Fetal bovine serum (FBS), penicillin (100 µg/ml), and streptomycin (100 µg/ml) in CO₂ incubator at 37°C.

Preparation of Plant fruit extracts:

The powdered fruit (500g) was repeatedly extracted in a 5L round bottomed flask with 2L of solvents in the order, ethyl alcohol (78°C), methanol (60°C), hexane (80°C), ethyl acetate (60°C-80°C) and finally water (100°C). Before extracting with next solvent the residue is powdered, dried in an air oven below 50°C and finally kept in water for 24 h to obtain the aqueous extract. The extract was concentrated by distilling off the solvent and then evaporating to dryness on a water-bath. The extracts were cooled at room temperature, and evaporated to dryness under reduced pressure in rotary evaporator.

Antiproliferative activity of *Coccinia grandis* on HEPG-2 cell lines:

The antiproliferative activity of the ethyl acetate extract of *Coccinia grandis* against HEPG-2 cell lines was determined by methylthiazolyl diphenyl- tetrazolium bromide (MTT) assay [11]. In a 24-well plate (Costar Corning, Rochester, NY), the cells were allowed to grow for 48 hours to reach confluence. The cells were then incubated in the presence of various concentrations of the *Coccinia grandis* ethyl acetate extract in 0.1% DMSO for 48h at 37°C. After removal of the plant extract solution and washing with phosphate-buffered saline (pH 7.4), 200µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromide cells (MTT) phosphate-buffered saline solution was added. After 4h incubation, 0.04M HCl/ isopropanol were added. Viable cells were determined by the absorbance at 570nm. Measurements were performed and the concentration required for a 50% inhibition of viability (IC₅₀) was determined graphically. The absorbance at 570 nm was measured with a UV- Spectrophotometer using wells without sample containing cells as blanks. The effect of the samples on the proliferation of HEPG-2 was expressed as the % cell viability, using the following formula:

$$\% \text{ cell viability} = (A_{570} \text{ of treated cells} / A_{570} \text{ of control cells}) \times 100$$

DNA Fragmentation:

For the DNA fragmentation assay [12], 0.5 ml of the cell suspension was centrifuged and the pellet was collected and dissolved in TTE (Tris-Taps-EDTA) buffer with vigorous shaking. The mixture was centrifuged and the supernatant was transferred carefully to separate tubes marked as T. To the pellet added 0.5 ml of TTE buffer, 0.5ml of Ice-cold 1M NaCl and 0.7 ml of ice-cold isopropanol. The mixture was incubated overnight at -20°C to enhance precipitation. The content was centrifuged further to obtain pellet and was purified by repeated wash with 0.5 ml of 70% ice cold ethanol. The final pellet obtained was air dried and dissolved in 20-50µl of TE (Tris-EDTA) buffer. The fragmentation patterns of both the HEPG-2 and MCF-7 cell lines were analyzed and compared with their control.

Results and Discussion:

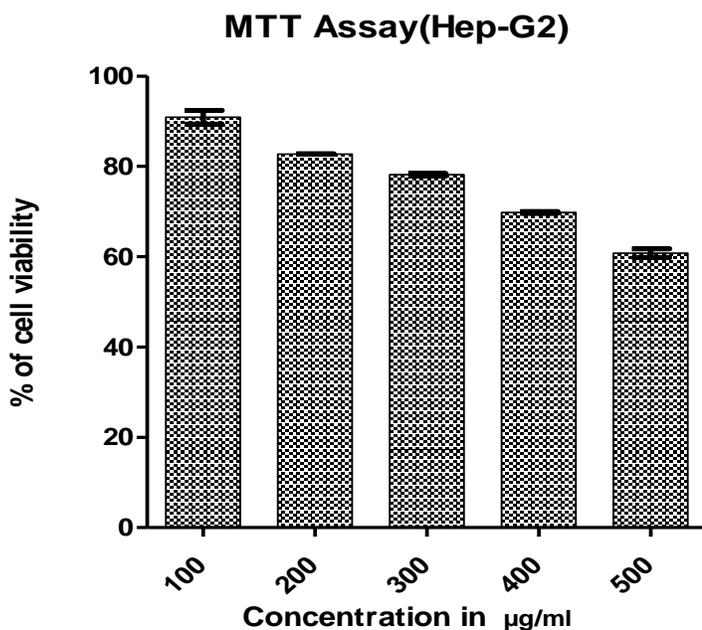
Effects of *C. grandis* extracts on cell viability:

The effect of *C. grandis* fruit extract (0.1 – 0.5 mg/ml) on cell viability of HepG2 cell line was determined (Table 1). It is seen that the viability of the cells decreased with the increase in concentration of the extract. At 0.1 mg/ml the extract showed 88.86 % viability which drastically decreased to 60.77 %. This shows that the fruit extract has the ability to decrease the cell division.

Table: 1 Cytotoxic activity of the fruit extract of *Coccinia grandis* (MTT assay) (mean \pm S.D.)

Tested concentration (mg/ml)	OD values at 570 nm
0.1 mg	1.889
0.2 mg	1.720
0.3 mg	1.624
0.4 mg	1.451
0.5 mg	1.264
Control	2.063

Tested concentration (mg/ml)	% of cell viability
0.1 mg	88.861
0.2 mg	82.732
0.3 mg	78.139
0.4 mg	69.793
0.5 mg	60.774
Control	100



Cancer is the main killer disease in most developed as well as developing countries, which is induced by oxidative stress. Hence antioxidants which are very effective in combating cancer need thorough search especially safer compounds from plant sources. Increased oxidative stress encountered in body due to either environmental hazard, or impairment in the body metabolism due to varying disease conditions including drugs or having insufficient amount of dietary antioxidants, has to be curbed by exogenous supply of antioxidants as a choice of therapy or preventive measure.

The high mortality rate of liver cancer and the absence of effective chemotherapy have demanded a continued need for new alternatives for treatment and prevention, and natural products play a dominant role in the discovery of such new drugs.

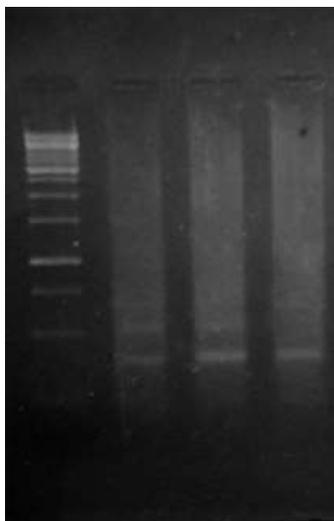
Assessment of the in-vitro cytotoxicity has recently become a primary screening method for evaluating the anticancer properties of various chemicals and natural substances. The cell viability (MTT assay) was investigated in *Coccinia grandis* fruit extract treated Hep G2 cell lines. Thus, the inhibitory activity of the extract provides evidence for the in vitro cytotoxicity. The present investigation confirmed the anti-proliferative, cytotoxic and apoptogenic ability of methanolic extract of *Coccinia grandis* fruit extract on HepG2 (Human liver carcinoma cell line).

The anti-proliferative and the cytotoxic activities of the various extracts of *C. grandis* have been supported by the observations in cell viability study and in MTT assay respectively. The extracts inhibited the growth and the metabolic activities of HepG2 cell line. The methanolic extract was found to show highest activity.

DNA Fragmentation analysis:

To determine the mechanism of cell death initiated by the plant extract, we employed the DNA fragmentation assay, a hallmark of programmed cell death. Induction of apoptotic DNA fragmentation by *C. grandis* extracts on HepG2 cell lines was studied. We found that the cells when exposed to 50, 100 and 150 μg underwent apoptosis. This was clearly observed as a fragmented DNA profile. The conventional agarose gel electrophoresis was performed in order to verify DNA fragmentation. Figure 2 shows a fragmented DNA ladder pattern and this was effected in a dose dependent manner. This indicates that the DNA might be cleaved after 24 hours of treatment. The fragmented DNA was observed around 5 to 8 kbp, which was smaller than the typical fragmentation of DNA at 20 to 300 kbp when entering early stage of apoptosis. The gel pattern of the DNA samples isolated from untreated control HepG2 cells showed intact DNA bands whereas the gel pattern of the cells treated with the extract confirms the cause of apoptosis in the cell line.

Fig 3.15: DNA fragmentation pattern of HEpG-2 cell lines



1 2 3 4

Lane 1 – 1Kb DNA ladder

Lane 2 –untreated HepG2 (Liver cancer) cell line

Lane 3 – active fraction 100µg treated HepG2 (Liver cancer) cell line

Lane 4 – active fraction 150µg treated HepG2 (Liver cancer) cell line

Apoptotic cells often produce a unique ladder composed of nucleotide fragments at an interval of 180-200 base pairs, which can be visualized by DNA-agarose gel electrophoresis. It is generally assumed that the toxicity of antitumor drugs is the consequences of their ability to cause genomic DNA damage in cancer cells [13].

Fragmentation of nuclear DNA extracted from apoptotic cells is a hallmark feature of apoptosis. That is, DNA cleavage during apoptosis occurred at sites between nucleosomes, protein-containing structures that occur in chromatin at 200-bp intervals. This DNA fragmentation was often analyzed using agarose gel electrophoresis to demonstrate a “ladder” pattern at 200-bp intervals. The ladder pattern of fragmentation in apoptosis might be a consequence of the state of the chromatin at the time of fragmentation. The detection of DNA fragmentation and the presence of single strand ends of DNA have continued to be an assay used in many studies to detect apoptotic cells, particularly in intact tissues. This is in spite of the fact that necrosis also produces single-strand DNA ends in cell nuclei.

References:

- [1] AK Mukherjee; S Basu; N Sarkar; AC Ghosh. *Curr Med Chem*, **2001**, 8, 1467–1486.
- [2] DM Eisenberg; RB Davis; SL Ettner; S Wilkey; M Van Rompay; RC Kessler. *J Am Med Assoc*, **1998**, 280, 1569–75.
- [3] AV Russo; L Cardile; A Vanella and JA Garbarino. *J Ethnopharmacol*, **2001**, 100, 323–332.
- [4] P. Anand, A.B. Kunnumakkara, *Pharm. Res.*, **2008**, 25(9), 2097–2116.
- [5] P Joseph. *Facts on File Inc, New York*, **2004**, 42-54.
- [6] Douglas Hanahan, A. Robert. *Cell*, **2000**, 100, 57–70.

- [7] GM Cragg; DJ Newman. *Expert Opinion on Investigational Drugs*. **2000**, 9, 1–15.
- [8] A Ramos; AV Piloto; A Garlia; CA Rodriguez; R Rivero. *J Ethnopharmacol*, **2003**, 87, 241–6.
- [9] SMA Kawsar; E Huq and N Nahar. *Intl J Pharmacol*, **2008**, 4(4): 297-300.
- [10] Bhattacharya Bolay et al.. *Journal of Phytology*. 2010; 2(11): 52-57.
- [11]T. Mosmann. *J. Immunol. Methods*, **1983**, 65(1-2), 55-63.
- [12]Boon Chin Heng, Kumar Jayaseelan Vinoth, Kai Lu, Xuliang Deng, Zigang Ge, Boon Huat Bay, And Tong Cao. *Biocell*, **2007**, 31(3), 405-410.
- [13]. Jamieson ER, Lippard SJ. *Chem Rev*. 1999 Sep 8;99(9):2467-98.