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Anticancer Activity of *Cissus quadrangularis* L. Methanolic Extract against MG63 Human Osteosarcoma Cells – An in-vitro Evaluation using Cytotoxicity Assay

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To evaluate the anticancer effects of *Cissus quadrangularis* leaf extract against MG63 cells. MG63 cells were obtained from NCCS, Pune. The methanolic extract of *Cissus quadrangularis* was prepared and its anticancer activity was tested in cell lines using Mossman method of cytotoxicity assay. The cell viability of MG63 cells ranged between 29.65% and 73.59% at an extract concentration from 1000μg/ml to 7.8μg/ml. The IC_{50} of extract revealed by this cytotoxicity assay was around 100 μg/ml. This study showed anticancerous activity of *C.quadrangularis* leaf extract against MG63 cells, which can be further characterized by future studies and aid in treatment of bone tumors.

Keywords: Cancer, MTT assay, methanolic extract, bone tumor, *Cissus quadrangularis*.

Cancer is a disease with multistep and multiple factor pathogenesis, and a rising concern causing major health burden worldwide. According to the International Association of Cancer Registries (IACR – GLOBOCAN database), 12.7 million new cases of cancer, and 7.6 million deaths due to cancer were reported worldwide[1]. The numbers increased to 14.1 million new cases and 8.2 million cancer deaths by 2012[2]. The recently updated database in 2018 has reported new cancer cases of 18.1 million, and cancer deaths of 9.6 million, which proved the mounting burden of cancer incidence as well as mortality[3]. In United States, bone carcinomas constitute 0.2% of all cancer conditions. Current treatment modalities for bone malignancies include surgeries, chemotherapy, radiotherapy, as well as immunomodulation which comprise of high mortality risk. This implicates an urgent need for new treatment strategies with fewer side effects to effectively combat malignant conditions[4]. During the last five to six decades, clinical applications of plant products, metabolites, and their derivatives have been effectively introduced into the armamentarium to fight against cancer[5]. More number of studies has been done to evaluate anticancer activity of plant samples, as well as plant extracts, and many of them successfully entered the market worldwide for treatment of cancer[6]. Thus it is imperative to search for novel plant phytoconstituents which possess the ability to fight against cancer cells. Plant phytoconstituents rich in antioxidants were known to reduce cancer mortality, and increase life expectancy[7,8].

Cissus quadrangularis. Linn is a perennial plant which belongs to Vitaceae (grape) family. It was reported to be native of India and Africa. The

plant has been known by many names according to the geographical area. Some of the common names are Kandvel, Perandai, Asthisamdhani, Hadjod, Harbhanga, Varavalli etc. Almost all parts of the plant including stem, leaves, as well as roots are being used as medicine. The medicinal uses of Cissus quadrangularis were realized and well documented in native medicine including siddha, ayurvedha. The pharmacological properties were extensively studied in literature and of wide spectrum. The spectrum includes anti-inflammatory, analgesic, antitumor, antiosteoporotic, antibacterial, anticonvulsant, antipyretic, antifungal, antidiabetic, gastroprotective, and hepatoprotective [9,10,11]. The phytochemical constituents of the plant have been characterized. The stem contains calcium, phosphorous which helps in bone formation. Some of the important constituents are amyrins (α,β), carotene, vitamin C, β-sitosterol, resveratrol, flavonoids such as quercetin, quadrangularins (A,B, C), and kaempferol[12]. MG63 cells were derived from human osteosarcoma, which were well characterized, and able to provide better understanding to study anticancer activity against bone tumours[13]. With this background we undertook an in-vitro study to investigate anticancer activity of *C.quadrangularis* methanolic extract against MG63 cells.

MATERIALS AND METHODS

Plant Collection and Extract Preparation

Cissus quadrangularis plants were collected from localities around Chennai, Tamilnadu. The authentication was done by the Botanist Prof. V. Chelladurai, Central Council for Research in Ayurvedha and Siddha, Government of India. The aerial parts of the plants were dried up in shade and then the dried parts were powdered. The powder was subjected to methanolic extraction using Soxhlet apparatus[14].

Cell Culture

Human MG63 cell lines were procured from the Cell repository of National Centre for Cell Sciences (NCCS), Pune, India. Dulbecco's Modified Eagle Media (DMEM) was used for maintaining the cell line, which was supplemented with 10% Fetal Bovine Serum (FBS). Penicillin (100 U/ml), and streptomycin (100 µg/ml) were added to the medium to prevent bacterial contamination. The medium with cell lines was maintained in a humidified environment with 5% CO_2 at 37 °C.

Cytotoxicity assay

The MG63 cells were placed in 24 well plates (1 X 10^5 cells per well) and incubated in 5% CO_2 environment at 37°C. Cells (1 × 10^5/ well) were placed in 24-well plates and incubated in 37^0C with 5% CO_2 condition. Once the cells placed in wells reached confluence, the prepared concentrations of extract from 1000µg/ml to 7.8µg/ ml were added and kept in incubator for 24 hours. Then the samples were removed from the well, and washed with phosphate-buffered saline (pH 7.4) or DMEM without serum. 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl—tetrazolium bromide (MTT) was added to each well (100 µl/well) and incubated for 4 hours. Then 1ml of dimethyl sulfoxide (DMSO) was added in all the wells to dissolve the formed formazan crystals. Each sample was placed in the cuvette; using DMSO as the blank the absorbance value at the wavelength of 570 nm was noted using UV-Spectrometer. The average absorbance values from three observations were taken. The observed values were tabulated, and the concentration required for 50% inhibition (IC_{50}) was determined graphically. The percentage cell viability was calculated by determining the ratio between A570 of treated cells, and A570 of control cells, multiplied by 100. Cell control and sample control is included in each assay to compare the full cell viability assessments[15].

RESULTS

The methanolic extract of *C.quadrangularis* showed cytotoxicity against MG63 cells in concentration dependent manner [Figure 1]. IC_{50} (half maximal inhibitory concentration) revealed by the assay was around 100 µg/ml at a dilution of 1:4. The MG63 cell viability with various concentrations of *C.quadrangularis* leaf extract was tabulated in table 1. The cell viability of MG63 cells ranged between 29.65% and 73.59% at extract concentrations of 1000 µg/ml and 7.8 µg/ml correspondingly. The cell viability and the cytological characteristics of MG63 cells were depicted in Figure 2.

DISCUSSION

The anti-proliferative properties of the *C.quadrangularis* methanolic extract from aerial parts against MG63 cells were shown in this study using cytotoxicity assay. The assay detects the reduction of dimethylthiazole diphenyl tetrazolium bromide (MTT) salt to a coloured formazan product by mitochondrial enzyme succinate dehydrogenase, the intensity of the colour was measured using spectrophotometer, which measures the quantity of viable cells[15, 16]. The cell viability of MG63 cells decreased with increase of extract dose confirming the anti-cancerous property of the extract with IC_{50} value at around 100 µg/ml. Several naturally derived plant products with prospective anticancer properties against MG63 cells have already been reported by other authors[17,18] and the reported IC_{50} values of other plant extracts [19-21] compared to this study results suggest that *C.quadrangularis* methanolic extract exhibits considerable inhibition of MG63 cells.

In an in-vitro study both chloroform as well as ethanol extract from the leaves of Cissus quadrangularis were compared, and studied for their anti-oxidant and anticancer activity. Ethanol extract was found to be better than chloroform extract for both the properties. The extract also showed potent anticancer activity against Ehrlich Adenocarcinoma cell lines which was demonstrated by MTT assay as well as tryptan blue method. The mechanism of cytotoxicity was postulated to be due to membrane damaging effect, and activation of apoptotic pathways[22].

Table 1. Cell viability of MG63 cells treated with *Cissus quadrangularis* methanolic extract

S. No	Extract concentration (µg/ml)	Dilution	Absorbance at 570 nm	% Cell viability
1	1000	Neat	0.602	29.65
2	500	1:1	0.721	35.51
3	250	1:2	0.828	40.78
4	125	1:4	0.989	48.71
5	62.5	1:8	1.112	54.72
6	31.2	1:16	1.245	61.33
7	15.6	1:32	1.344	66.20
8	7.8	1:64	1.494	73.59
9	Cell control	-	2.030	100

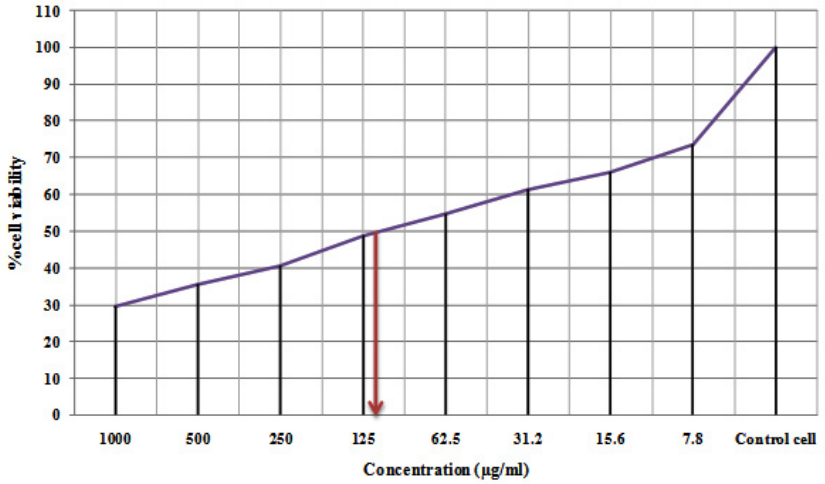
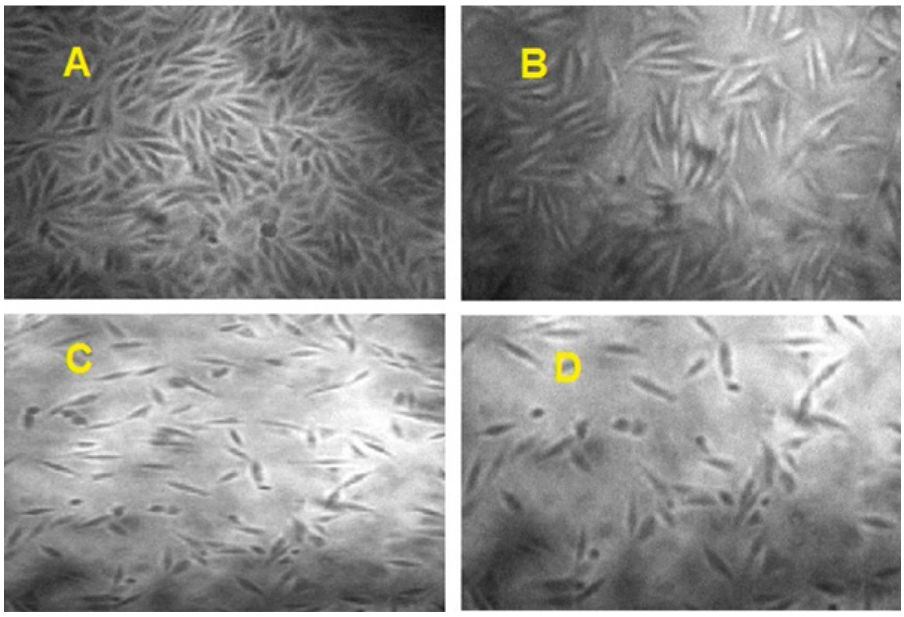


Fig. 1. MTT assay - IC_{50} of *Cissus quadrangularis*



A- Control cells, B- Cell viability at 7.8 µg/ml, C- Cell viability at 125 µg/ml, D- Cell viability at 1000 µg/ml.

Fig. 2. Anticancer activity of *Cissus quadrangularis* methanolic extract on MG63 osteosarcoma cells

The anticancer activity of alcoholic extract of the plant also have been demonstrated with various cell lines including HeLa (Cervical cancer), KB (Oral epidermoid carcinoma cell line), A431 (Skin epithelial carcinoma cell lines), MCF7 (Breast cancer cell line), HEp 2 (Human laryngeal carcinoma), HT29 (Colon carcinoma), and Vero cell line (Kidney epithelial cell). Inducing the production of reactive oxygen compounds in cancer cells, arresting the cell cycle at G1 phase by apoptosis activation were the possible proposed mechanisms of anticancer action[23-25].

MG-63 osteosarcoma cell lines were utilized as a tool to assess the anticancer potential of molecules against skeletal malignancies. In a study conducted by Wang Jun 2017, the researchers demonstrated the anticancer activity of curcumin against MG-63 osteosarcoma cells. Considering the fact that these cells had p-53 mutation, which has led to uncontrolled proliferation, the anticancer effect of curcumin was considered to be by the action over the p-53 signalling pathway[26].

The use of *Cissus quadrangularis* in the management of bone and joint disorders such as osteoporosis, osteoarthritis, and rheumatoid arthritis has been documented in native medicine. The antiosteoporotic potential of the ethanolic extract has been observed effectively in rat model of osteoporosis. The possible mechanism of this activity was attributed to its ability to enhance the differentiation of mesenchymal stem cells to osteoblasts, therefore enhancing bone formation. Wnt-â catenin pathway could be the target through which the plant extract exert its osteogenesis action[27]. The same mechanism can have a contributing role in the anticancer action against osteosarcoma too.

CONCLUSION

With the results of the present study, it may be inferred that methanolic extract of *Cissus quadrangularis* possess therapeutic potentiality against bone tumours. Further studies can be done on this plant extract to obtain more data for characterisation of responsible anticancer phytoconstituents, potential mode of action, as well as take the research forward for further exploration.

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