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REVIEW ON EFFECT OF CISSUS QUADRANGLARIS ON GLIOBLASTOMA, OSTEOSARCOMA, CERVICAL CANCER CELLS AND ON SKIN CANCER CELLS

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ABSTRACT

Cissus Quadrangularis is a perennial plant of the grape family. It is used in the human population with various objectives of treatment in obesity, fractures, joint pain, low bone mass. It has recntly demonstrated the anti – cancer effect by significantly inhibiting tumour growth and inducing cell death. In the different types of cancer cells observed; in glioblastoma cells it inhibits the cell viability and causes mitochondrial fragmentation in U87 cells, the study on osteosarcoma cells showed its efficacy as an anti-proliferative effect, study on skin cancer cells compared the anti-activity of Active fraction of cissus Quadrangularis with that of the standard. The studies was mainly done on the basis of different assay techniques.

KEYWORDS: Cissus Quadrangularis, Glioblastoma, Osteosarcoma cells.

INTRODUCTION

- Natural products from plants are undeniably a vital resource to explore the possible lead compounds for the treatment of cancer.
- During the last five to six decades, plant products, metabolites, and their derivatives have been effectively introduced into the armamentarium to reduce the replication of cancer cells. Plant phytoconstituents are rich in antioxidants which were known to reduce cancer death rates and increase life expectancy.
- Cissus quadrangularis (CQ) commonly known as Hadjod, is a succulent plant of the family *Vitaceae* belonging to the class of *Magnoliopsida* native to south Asia and Africa containing phytosterols, anabolic steroidal substance, ascorbic acid, triterpenoids, vitamin E, beta carotene and calcium. Almost all parts of the plant including the stem, leaves, as well as roots are being used as medicine.
- It poses as a promising safe chemo-preventive plant to combat cancer.
- Indications for the plant extract Cissus Quadrangularis finding its use in the treatment of osteoporosis, osteoarthritis, helminthiasis, digestive diseases, diabetes, skin, eye, and ear diseases. The pharmacological properties of the plant extract are linked to cell reinforcement, free radical search, hostility to microbial, bone regeneration, ulceration, pain relief, mitigation, and diuretics.
- Recent studies have shown that Cissus Quadrangularis showed a significant antioxidant and anti-cancer activity against the Ehrlich Ascites Carcinoma cell line.

AIMS AND OBJECTIVES

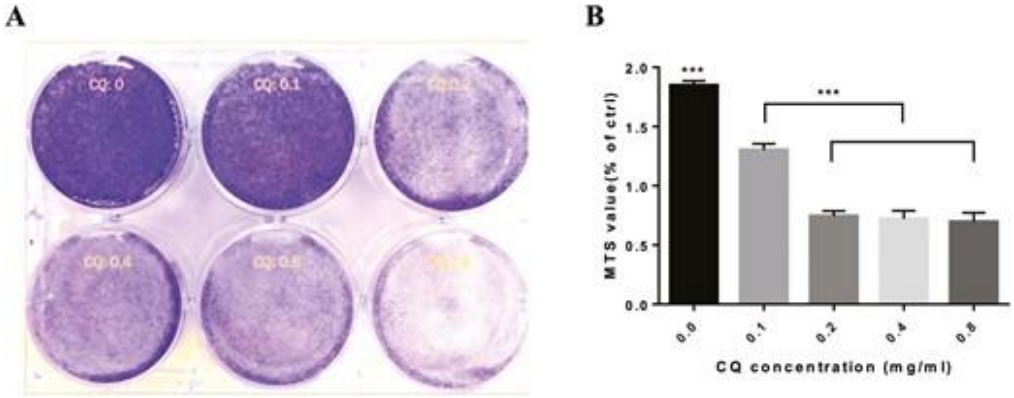
- Though the study is being performed in the four different cells taken from different cell lines of humans done to evaluate the anti-cancer effect of Cissus Quadrangularis
- To evaluate the chemotherapeutic potential of Cissus Quadrangularis in treating the brain tumor glioblastoma multiforme.
- To study the anti–proliferative properties of Cissus Quadrangularis methanolic extract from aerial parts against MG63 cells are assayed using the method of cytotoxicity assay.
- To evaluate the anti-cancer activity of Cissus Quadrangularis by taking its extract i.e; active acetone fraction of Cissus Quadrangularis (AFCQ), and by comparing its effect with standard anti-cancer antibiotic Doxorubicin which is done by evaluating the status of apoptotic markers.
- Also to evaluate the Bax-Bcl-2 ratio along with the release of cytochrome C from mitochondria to the cytoplasm, which is considered to be the hallmark of the apoptosis process.

MATERIALS AND METHODS

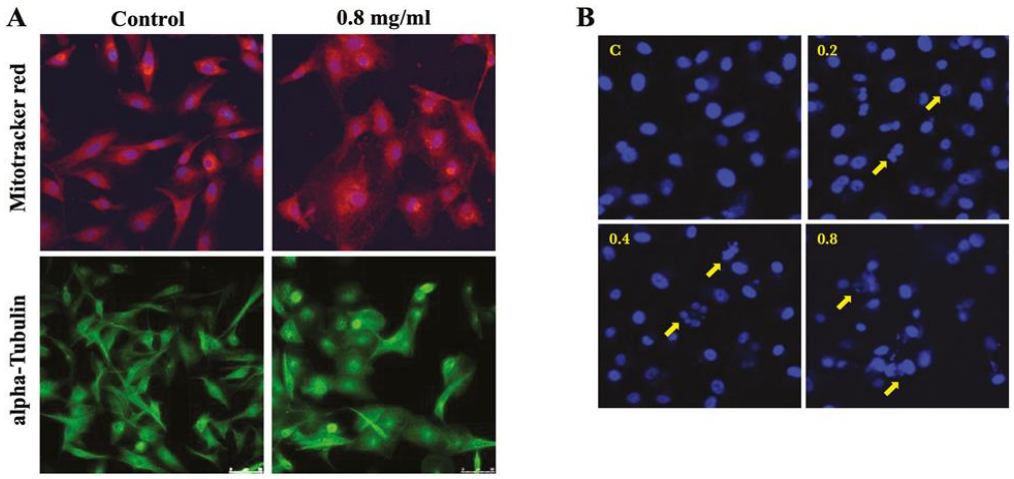
- The materials used for the *first study* include the reagents like ethanol –extracted Cissus Quadrangularis. Also, the cell culture includes the glioblastoma cell line (U87) was purchased from the American type culture association. Different assay techniques were used in the study cell proliferation and viability assays, wound healing assays, mitochondrial membrane potential (MMP) visualization method, Mitotracker staining, cell morphology and immunocytochemistry, DNA damage, and cell apoptotic determination, western blot analysis and Statistical analysis.
- *Second study* includes plant collection and extract preparation of aerial parts of the plant (Cissus Quadrangularis). Cell culture in this study was done like human MG63 cells was procured from the cell repository of the national center for cell sciences. The method used was a cytotoxicity assay.
- In the *third study* conducted cell viability was analyzed in HeLa cells at different concentrations (25-300µg/ml) of CQ extract. Reactive oxygen species(ROS) generation, cellular apoptosis, cell cycle analysis, and caspases-3 activity were evaluated. In silico, structure-based virtual screening analysis was carried out using Auto Dock Vina and iGEMDOCK.
- *Fourth study* was carried out on A431 human cell line (skin epidermoid carcinoma). The A431 cells were treated with five different extracts (Hexane, chloroform, ethyl acetate, methanol, and acetone) of Cissus quadrangularis in a dose-dependent manner to evaluate the anti-cancer activity of CQ.

RESULTS

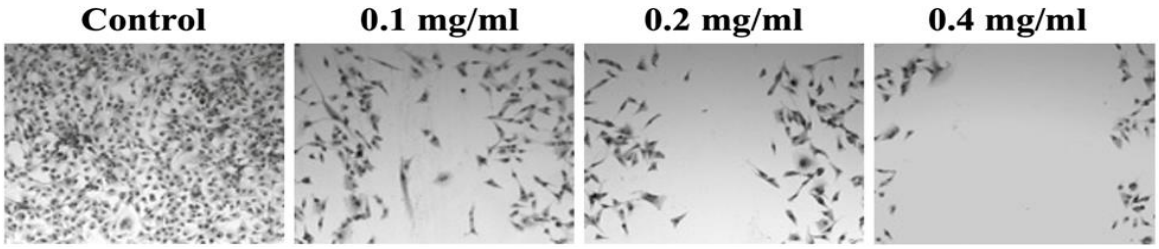
- The first glioblastoma cell investigation found that Cissus quadrangularis caused cell cycle arrest, cell death, and cytotoxicity in U87 cells. Additionally causes cytoskeletal deformation in U87 cells and reduces the viability of glioblastoma cells.



Cissus chloroquine (CQ) inhibits U-87 cell proliferation/viability. (A) Cells were treated with various concentrations (0, 0.1, 0.2, 0.4, 0.8, 1.6 mg/ml) of CQ as indicated for 48 h and then stained with Crystal Violet. (B) Cells were treated with various dose of CQ for 48 h. MTS assay was carried out using MTS assay.



CQ causes mitochondrial fragmentation, cytoskeletal deformation and nuclear condensation in U-87 cells. (A) Control and 0.8 mg/ml of CQ. U-87 cells were treated for 24 h, then stained with Mitotracker red and alpha-Tubulin according to the protocol. The nucleus appears in blue. (B) Cells were treated with various concentrations of CQ (mg/ml) for 24 h. Cells were then stained using Hoechst staining and photographed under fluorescence.



CQ inhibits U-87 cell migration in a dose-dependent manner. Lines of equal width were scratched into similar dishes of cells which were then treated with various concentrations of CQ for 24 h and then stained with Haematoxylin/Eosin for clarity.

- The Mossman method of cytotoxicity was used in the second investigation on osteosarcoma MG63 cells to assess the cytotoxicity of different doses of Cissus quadrangularis leaf extract. The microscopic images of the cells treated with the extract are presented in the figure, and they allow us to see the cell viability and cytological properties of MG63 cells. The quantity of viable osteosarcoma cells and the structure of those cells have been drastically decreased by exposure to higher concentrations of the 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

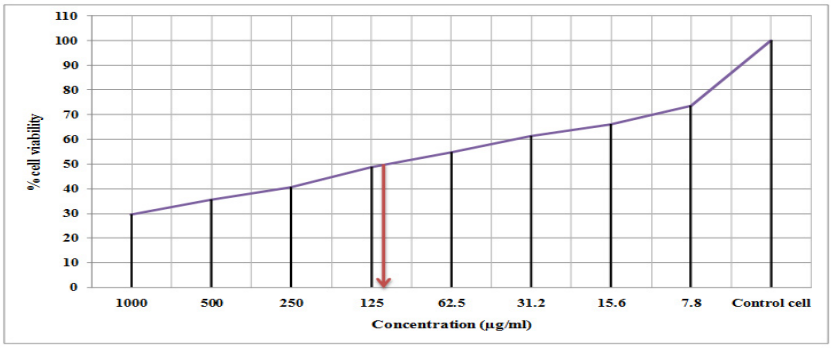
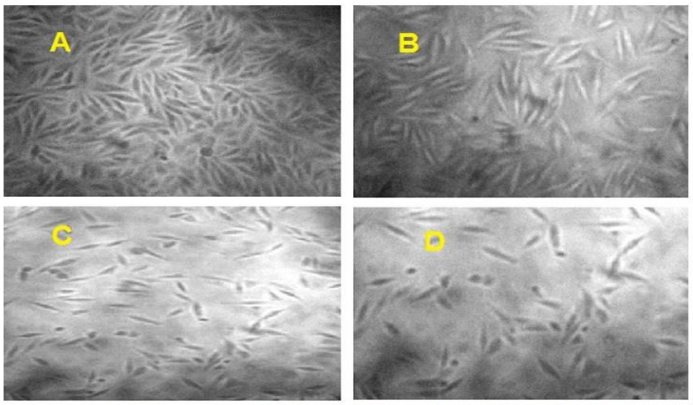


Figure 1: MTT assay - IC_{50} of *Cissus quadrangularis*.

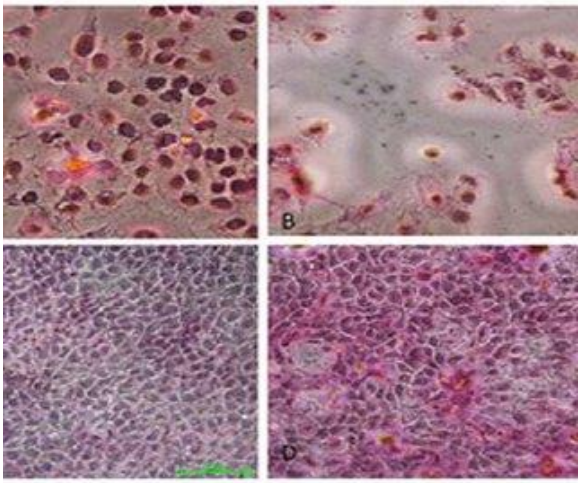


A- Control cells, B- Cell viability at 7.8 µg/ml, C- Cell viability at 125 µg/ml, D- Cell viability at 1000 µg/ml.

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

- In the ***third study*** done on cervical HeLa cell line, cell viability of these cells was reduced significantly ($p<0.05$) in a dose-dependent manner, however Cissus Quadrangularis showed non-toxic to normal kidney epithelial NRK-52E(Normal rat kidney tubular epithelial cell line (NRK-52E), Cissus Quadrangularis extract induced the intracellular reactive oxygen species level, nuclear condensation and reduced the mitochondrial membrane potential (MMP),with the induction of annexin V-FITC positive cells. Cissus Quadrangularis extract arrested cells in the G0/G1 and G2/M checkpoints and activated caspase -3 activity significantly in HeLa cells. The molecular docking study showed a strong binding affinity of Cissus Quadrangularis phytocomponents against the caspase -3 (PDB-ID: 1GFW) protein of human apoptosis. PASS Analysis of selected active components using Lipinski's rule of five showed promising results. Further, drug-likeness and toxicity assessment using OSIRIS (Open source STR analysis software) Data warrior V5.2.1 software exhibited the feasibility of phytocomponents as drug candidates with no predicted toxicity.

The ***fourth study*** shows the measuring of anti-cancer activity on A431 skin epidermoid carcinoma cell line, in which the A431 cells were treated with different extracts of CQ, in a dose – dependent manner. Hexane, Chloroform, ethyl acetate, methanol and acetone were the extracts used and exhibited cytotoxicity but to a lesser extent to that of acetone extract which showed the potential of anti-cancer activity of Cissus Quadrangularis in A431cell line. The G1 (50) value of the acetone extract was found to be 8µg ml (-1), whereas GI (50) purified fraction of acetone extract, termed as AFCQ (active acetone fraction of CQ) with respect to A431 cells, was found to be 4.8 µg ml (-1). Along with this the mechanism of anti-cancer activity of Cissus Quadrangularis demonstrated by AFCQ was investigated by comparing its effect with the standard anticancer drug Doxorubicin (DOX) and evaluating the status of apoptosis markers after treatment of A431 cells with AFCQ and Doxorubicin. Determination of Bax-Bcl-2 ratio along with the release of cytochrome c from mitochondria to cytoplasm, which is a remarkable step in the process of apoptosis was also done. Cleavage of PARP revealed that AFCQ induces apoptosis in A431 cells with reference to Doxorubicin.



ıtrol, B- Cancer Treated, C-normal control, D- nc

CQ significantly altered the morphology of HeLa cancer cells (Figure 1B) as compared to control; however at the same dose of CQ extract, normal skin cells (HaCaT) does not show morphological alteration (Figure 1D). (Figure 1A) is HeLa control and (Figure 1C) are HaCaT control cells. Simultaneously the cell density is also low in HeLa treated section Figure.1B in comparison to normal treated, normal control and cancer control section Figure 1D, 1C and 1A respectively.

DISCUSSION AND CONCLUSION

- Cissus Quadrangularis is a perennial herb from the grape family. It has been used for its healing properties in the repair of bone fractures and injured tendons. Recently CQ has been reported to have potential anticancer activity. CQ is observed to cause DNA fragmentation and cell death in the HeLa cell line. Previously it was reported that CQ was able to promote osteoblast differentiation in a dosage-dependent manner. CQ could induce A431 cell death via mitochondrial-mediated apoptosis by increasing the mitochondrial membrane's permeability.
- The study conducted on U87 MG glioblastoma cells demonstrated the cytotoxic and anticancer effects. Treatment with CQ had shown many mechanisms of it such as inhibition of pre-survival signaling pathways, induced apoptosis, and cell cycle arrest, reduced cell viability, inhibited cell migration, and caused changes in MMP and cell/nuclear morphology. Considering all the above-mentioned findings, it can be concluded that CQ may serve as a potential lead compound for the development of future anticancer therapies.
- **The** anticancer activity of alcoholic extract of the plant also has been demonstrated with various cell lines that were utilized as a tool to assess the anticancer potential of molecules against skeletal malignancies.
- Even though the phenolic constituent of the plant can be considered as the antiproliferative factor for the cytotoxicity observed, chromatographic

characterization and quantification of the bioactive compounds present in the extract will broaden our knowledge and help us to optimize the compound for future research initiatives.

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