Cytotoxic Activity of Methanolic Extract of *Berberis aristata* DC on Colon Cancer

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Abstract: Natural products represent a reservoir of diverse templates and are being tapped to outsource novel anticancer agents. *Berberis aristata* DC (Fam: Berberidaceae) has been reported to be useful for the treatment of inflammation, cuts, wounds, eye and skin diseases, diarrhea, ulcers, cancers. In the present study, the methanolic extract of the stems of *Berberis aristata*, was investigated against human colon cancer cell line (HT29) to explore its anticancer potential. In addition, a phytochemical screening of the methanolic extracts was also done. The effect of *Berberis aristata* methanolic extract on proliferation of HT29 cancer cell line was determined by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) microculture tetrazolium viability assay. The cells were exposed to different concentrations (100, 50, 25, 12.5, 6.25, 3.125 and 1.5 µg/ml) of *Berberis aristata* methanolic extract or vehicle for 72 h. Cisplatin (5, 2.5 and 1.25 µg/ml) acted as positive control and vehicle (DMSO) as negative control. Following treatment, the cells were exposed to Tetrazolium dye (5mg/ml) for 4 h. The formation of the purple coloured formazan complex was dissolved by adding DMSO (100 µl) and read at 490nm using ELISA microtiter plate reader to determine the inhibitory concentration, IC$_{50}$. About 40% increment in cell killing was seen when the dose of *Berberis aristata* methanolic extract was increased from 1.5 to 25 µg/ml. At a concentration of 100 µg/ml, 54.89% cytotoxicity was recorded. The IC$_{50}$ value of *Berberis aristata* methanolic extract was 1.8964 µg/ml after 72 h of incubation. In this study, it was observed that *Berberis aristata* methanolic extract induces a concentration dependent inhibition of HT29 cells, with an IC$_{50}$ value of 1.8964 µg/ml after 72 h of incubation. The result of the phytochemical screening of the investigated methanolic extract of stems of *berberis aristata* showed the presence of alkaloids as active chemical constituents.

Key words: Anti-cancer agent • *Berberis aristata* • Cisplatin • MTT • Cytotoxicity • IC$_{50}$

INTRODUCTION

Cancer causes significant morbidity and mortality and is a major health problem worldwide. The incidence of colon cancer is rising in every country of the World. It is the fourth most common cause of cancer death (after lung cancer, stomach cancer and liver cancer). Thus, colon cancer is a worldwide disease and needs to be addressed seriously. Medicines derived from plants have played a pivotal role in health care of ancient and modern cultures. Ayurveda, the Indian system of medicine mainly uses plant based drugs or formulations to treat various ailments including cancer. Almost 60% of drugs approved for cancer treatment are of natural origin. Vincristine, etoposide, taxanes and camptothecines are all examples of plant-derived anticancer compounds [1]. Therefore, there is an urgent need to develop alternative therapeutic measures against this deadly disease. There is always the hope that the search among the traditional medicinal plants may provide potent and safe medicines. Significant steps have been taken by the WHO to carryout research on plants, that work having greater activity and few or no side effects.
**Berberis aristata** DC (Syn. Indian barberry; Daru haldi, Sanskrit: “daruharidra”) belongs to the family Berberidaceae. This plant has been traditionally useful in all types of inflammations, ENT infections, wound healing, dysentery, indigestion, uterine and vaginal disorders. It is well known for its anti-inflammatory and immunopotentiating property. Berbamine effectively inhibits chemically-induced hepatocarcinogenesis. Preliminary reports indicate that it possesses anticancer activity as tested against mouse leukemic L1210 cells, human hepatoma cells and colon cancer cells. It is postulated that its anticancer activity may be due to its COX-II inhibitory property [2]. The other uses of *Berberis aristata* are as cooling laxative diaphoretic, laxative and useful in rheumatism. The dried extract of the roots are applied externally to the eyelids to cure ophthalmia and other eye diseases [3]. It is also reported to be a mild laxative, antihepatotoxic, [4] anti-inflammatory, [5]. a tonic and is useful in curing ulcers and fevers (6). The chief constituent of *Berberis aristata* reported is barberine, which is a bitter alkaloid [7-9].

Many other plants have been examined to identify new and effective anticancer compounds, as well as to elucidate the mechanism of cancer prevention. The present study aimed to evaluate the possible cytotoxic activity of the *Berberis aristata* used in the treatment of several diseases, but with no reports on its inhibitory effect on colon cancer potential. Therefore, the aim of the present study was to evaluate the preliminary phytochemical screening and to screen the anticancer activity of *Berberis aristata* methanolic extract on human colon cancer cell line HT29.

**MATERIAL AND METHODS**

**Authentication of Herb:** *Berberis aristata* (stems) was purchased from authenticated herbal supplier, New Delhi, Authentication of the herb was carried out from the Department, National Bureau of Plant Genetic Resources (NBPRG), Pusa, New Delhi, India, where a specimen is conserved with the voucher No.NHC/NBPRG/2008/3.

**Berberis aristata Methanolic Extract Preparation:**

The stems of *Berberis aristata* was grounded into powdered form using an electrical mill. Extraction of the powder (70 gm) was done by soxhlet apparatus by using methanol (200 ml) for 72 hrs. All extract were evaporated to dryness by hot water bath and centrifuged at 4000 rpm at 20°C for 10 min to obtain the supernatant. Then it was reconstituted in distilled water and stored at-20°C. At use, the frozen extracts were thawed until they reached room temperature.

**Cell Culture:** Human colon cancer cell line (HT29) obtained from the AIIMS, New Delhi, India. HT29 cancer cell line were cultured in Dulbecco's Modified Eagle Medium (DMEM). All culture media were supplemented with 10% fetal bovine serum (FBS), 1% antibiotic and antimycotic solution (50,000 units/L of penicillin and 50 mg/L of streptomycin) and 2mM glutamine. Cultures were held in 75 cm² culture flasks at 37°C, 5% CO₂ and 95% relative humidity, changing media at least twice a week. [10].

**MTT Assay:** Cytotoxic activity of *Berberis aristata* methanolic extract against human colon tumor cell line was tested, using the microtitration colorimetric method of MTT reduction (Mosmann, 1983). The tetrazolium salt 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) is used to determine cell viability in assays of cell proliferation and cytotoxicity. MTT is reduced in metabolically active cells to yield an insoluble purple formazan product. Cells were harvested from maintenance cultures in the exponential phase and counted by a hemocytometer using trypan blue solution. The cell suspensions were dispensed (100 µL) in triplicate into 96-well culture plates at optimized concentrations of 1.5 x 10⁵ cells/ml for HT29, after a 24-hr recovery period, the Cisplatin standard or *Berberis aristata* methanolic extract was diluted with distilled water were added. Seven dilutions of *Berberis aristata* methanolic extract were tested (100, 50, 25, 12.5, 6.25, 3.125 and 1.5 µg/ml) with an incubation period of 72 h. Cisplatin was used as positive control and vehicle (DMEM) as negative control. For median inhibition concentration (IC₅₀) determination, dose-response curves were conducted with a series of different concentrations of *Berberis aristata* methanolic extract that were approximately equal to the IC₅₀. To control wells, only culture medium (100 µL) was added. After an additional 72 h incubation period, the medium in each well was aspirated and replaced with 20 µL of MTT working solution (MTT) stock solution mixed with medium to attain a final concentration of 0.5 mg/ml. MTT powder was dissolved in Dulbecco's PBS to form a stock solution of MTT (5 mg/ml). The stock solution was filter-sterilized through a 0.22 µm filter and stored at-20°C. The cells were incubated at 37°C for 4 h and then the medium was aspirated and replaced with 100 µL DMSO to dissolve the
formazan crystals formed. The culture plates were shaken for 5 min and the absorbance of each well was read at 490 nm with 655 nm as the reference wavelength.

The relative viability of the treated cells as compared to the control cells was expressed as the % cytoviability, using the following formula:

\[
\% \text{ Cytoviability} = \frac{[A_{490} \text{ of treated cells}] \times 100}{[A_{490} \text{ of control cells}]}
\]

\(\text{IC}_{50}\) was then determined by nonlinear regression analysis of the corresponding dose response curve.

**Calculations and Statistics:** Experiments were performed in six replicates. Results were expressed as percentage growth inhibition of control. \(\text{IC}_{50}\) values were derived from a nonlinear regression model (curvefit) based on sigmoidal dose response curve (variable) and computed using Graphpad Prism version 3.00, Graphpad Software. Data were expressed as mean ± S.E.M.

**RESULTS AND DISCUSSION**

The results of the phytochemical screening of the investigated methanolic extract of stems of *Berberis aristata* showed the presence of alkaloids and absence of flavonoids and terpenoids.

The *in vitro* screening of the methanolic extracts of *Berberis aristata* showed potential cytotoxic activity against the colon cancer cells. The results obtained are shown in Table 1.

About 40% increment in cell killing was seen when the dose of *Berberis aristata* methanolic extract was increased from 1.5 to 25 µg/ml. At a concentration of 100 µg/ml, 54.89% cytotoxicity was recorded. The \(\text{IC}_{50}\) value of *Berberis aristata* methanolic extract was 1.9648 µg/ml after 72 h of incubation. In this study, it was observed that *Berberis aristata* methanolic extract induces a concentration dependent inhibition of HT29 cells, with an \(\text{IC}_{50}\) value of 1.9648 µg/ml after 72 h of incubation. The results obtained from the present study showed that *Berberis aristata* moderately cytotoxic activity.

**CONCLUSION**

*In vitro* cytotoxic activity against HT29 cell line at different concentrations were evaluated. Cytotoxic effect against HT29 colon cancer cell line is considered as a predictive anticancer activity indicator and \(\text{IC}_{50}\) value calculated for *Berberis aristata* methanolic extract was below 50 µg/ml, which indicates that *Berberis aristata* methanolic extract potentially present an interesting cytotoxic activity and should be evaluated against primary cultures to determine the selectivity of their effects. The cytotoxic activity may be due to the presence of alkaloids in the stems of *Berberis aristata* DC. The observed *Berberis aristata* methanolic extract activity results consider *Berberis aristata* as a potential anticancer herb against colon cancer. It also justifies the folklore medicinal uses and claims about the therapeutic values of this plant as curative agent and we therefore,

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<th>Cell line</th>
<th>Cytotoxicity of Berberis aristata methanolic extract against after 72 h incubation</th>
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<tr>
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<td>Dose(µg/ml)</td>
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<td>HT29 (Human colon cancer cell line)</td>
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Each value represents mean ± S.E.M. of six replicates (n=6).
suggest further, the purification and characterization of the phytochemicals along with investigations are needed to provide some additional insight into the *invivo* cytotoxic activity of the plants with a view to obtaining useful chemotherapeutic agent.

**ACKNOWLEDGEMENTS**

The authors would like to thank the All India Institute of Medical Science (AIIMS), New Delhi, India for providing the cancer cell lines, the Libraries of National Medical Library (NML), New Delhi, India and Central Drug Research Institute (CDRI), Lucknow, India, for their support and motivation in carrying out this work and National Bureau of Plant Genetic Resources (NBPGR), Pusa, New Delhi, India for the botanical verification and authenticating the plant material.

**REFERENCES**