# Anticancer Activity of Methanolic Extract of Berberis aristata in MCF-7 Human Breast Cancer Cell Lines

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Abstract—Breast cancer is second most common in women and accounts for 23% of all occurring cancers in women. At present, more than 60% of the chemotherapeutic drugs are developed from plants and their derivatives, which can be used for development of anticancer drugs. An Indian barberry, Berberis aristata has been traditionally used for the treatment of inflammation, skin diseases, ulcers and cancers. In present study, the methanolic extract of stems of B. aristata, was used to investigate its anticancer activity in human breast cancer cell line (MCF-7). Different concentrations of the methanolic extracts (125, 250 and 500 µg/ml) were subjected to determine the cytotoxic effect by measuring the cell proliferation activity in MCF-7 breast cancer cell lines up to 48 h of incubation. The IC<sub>50</sub> value for methanolic extracts identified was 220µg. Further, significant decreased (80%: p≤0.001) colony formation at 500µg/ml of methanolic extracts was noticed by soft agar assay in MCF-7 cells. However, in vitro scratch assay revealed the significant ( $p \le 0.001$ ) inhibition of cell migration up to 50% at 250µg of extracts. In addition, significant (68%) increase of apoptosis at 500 µg of extracts in MCF-7 cells was evidenced by live/dead assay.

*Index Terms—Berberis aristata*, Cytotoxicity, Cell migration, Apoptosis, Anticancer activity

# I. INTRODUCTION

Breast cancer is most common in women and more than 40% of all breast cancer cases are reported in developing countries. In India, breast cancer is the second most common cancer cause of death with 53,592 cases in 2008 (Parkin et al., 2000). Breast cancer causes due to various genetic and epigenetic alterations that affect the regulation and function of genes. In addition to the synthetic drugs, immense use of natural products and its derivatives in the development of anticancer drugs are increasing all over the world because of lesser side effects as compared to synthetic drugs (Newman et al., 2003, Srivastava et al., 2006, Gupta et al., 2010). As more than 60% of the chemotherapeutic drugs are developed from plants and their derivatives. Medicinal plants are potential sources of natural products exhibiting anti-proliferation and anti-metastatic properties (Sun et al., 2009).

B. aristata belongs to the family Berberidaceae (Parmar and Kaushal, 1982), also known as daruharidra and its extracts has been traditionally using in all types of inflammations, sensory organ infections, wound healing, dysentery, indigestion and uterine and vaginal disorders due to anti-inflammatory and immune-potentiating properties (Gupta et al., 2008). It is widely distributed throughout India and it has a large deciduous shrub with pale yellowish brown erect cylindrical twigs, elliptic spinous-toothed leaves, stalked flowers, small berry fruits, thick, woody, yellowish brown roots covered with a thin brittle bark (Ali et al., 2008). The extract of B. aristata roots has shown to be strong potential in regulating the glucose homeostasis through decreased gluconeogenesis and oxidative stress (Singh and Kakkar, 2009). In addition, the dried extract of roots of this plant was applied externally to the eyelids to cure ophthalmia due to it's a mild laxative property and extracts of fruits used as a tonic in curing ulcers and fevers (Janbaz and Gilani, 2000). Further, the methanolic extract of the stems of B. aristata, was investigated against human colon cancer cell line (HT29) to explore its anticancer potential (Das et al., 2009). In addition, it helps in significant reduction in serum cholesterol, triglycerides and low density lipoprotein levels and increase in thrombin and fibrinogen time in rabbits (Razzaq et al., 2011). It has been denoted that an active constituent of B. aristata, berbamine which is effectively inhibited the chemically-induced hepatocarcinogenesis (Gilani and Janbez, 1992). Further, it is postulated that COX II inhibitory activity of this compound may exerts the anticancer activity, as tested against mouse leukemic L1210 cells, human hepatoma cells and colon cancer cells (Fukuda, 1999). In addition to berbamine, other principal components present in the plant are berberine, oxyacanthine, aromoline and palmatine which exhibit multiple pharmacological activities (Fang et al., 2004, Rout, 2008, Nadkarni, 1989, Nayar, 2000, Parmar and Kaushal, 1982). Berberine has febrifugal, hypotensive, immuno-stimulating, antiinflammatory, antimicrobial antiprotozoal, anticholinergic, antiarrhythmic, antipyretic and antiamnesic activities (Soffar et al., 2001, Musumeci et al., 2003, Vaidya, 2006, Kulkarni and Dhir, 2007). Even though, the mechanism of action is not yet completely

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known in treatment of various inflammations or infections, it may assume that the active ingredients could be play role in mode of action. The present study aimed to evaluate the possible cytotoxic activity of the stems of *B. aristata* against human breast cancer cell line.

## II. MATERIALS AND METHOD

#### A. Plant Material and Methanolic Extract Preparation

*B. aristata* plants were collected locally and authenticated by a botanist, Ayurveda pharmacy, Tirupati, Andhra Pradesh. The shade dried stems of the plant was thoroughly grounded into powdered for using an electrical mill. The powdered form (80 g) of stem was extracted using methanol (200 ml) for 72 h by soxhlet apparatus. The extract was filtered through Whatman No.1 filter paper and evaporated in a rotarvapour at 40  $^{\circ}$ C to get completely dried form. The dried powder was transferred to sterile screw caps and stored at -20  $^{\circ}$ C. At use, the frozen dried extracts were dissolved in media.

#### B. Growth and Maintenance of Human Breast Cancer Cells

Human breast cancer cell line (MCF-7) obtained from the NCCS, Pune. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS), 1% antibiotic (50,000 units/L of penicillin and 50 mg/L of streptomycin) and 2 mM glutamine. Cultures were grown in 25cm flasks at 37 C, 5% CO<sub>2</sub> and 95% relative humidity, changing media at least twice a week.

## C. Cell Viability Analysis

Cell proliferation effect of *B. aristata* methanolic extract in the MCF-7 cells was determined by3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma, USA) as per method (Razzaq *et al.*, 2011). Briefly, monolayer cells of MCF-7 were trypsinized (0.25% trypsin, 10 min) and 5000 cells were seeded in 96-well plate. The cells were treated with desired concentrations of methanolic extract of *B. aristata* (125, 250, 500 and 1000  $\mu$ g) for 48 h at 37°C. Cell proliferation was evaluated by adding 10  $\mu$ l of MTT (0.5 mg/ml in PBS) to each well and incubated for 4 h. The medium was replaced with 100  $\mu$ L of 99.8% dimethyl sulphoxide in each well and optical density was measured at 570 nm in ELISA plate reader (Microplate reader, Biotek).

#### D. Soft Agar Colony Formation Assay

Soft agar colony formation assay was performed (Huang *et al.*, 2012) to determine the ability of cells for anchorage dependency. MCF-7 cells  $(10 \times 10^4)$  were plated in 60 mm culture plates and incubated overnight at 37 °C. The cells were treated with methanolic extract of *B. aristata* (125, 250 and 500 µg) at the indicated concentrations for 48 h. After treatment with drugs, the cells were harvested,  $0.4 \times 10^5$  cells were counted from each concentration by using trypan blue assay and the cells were suspended in 0.3% top agar (Difco, noble agar Detroit, MI) in the medium. Base agar (0.5%) was poured in 35 mm culture plates and top agar containing cells was

poured on the base agar. The cells were incubated at 37  $^{\circ}$ C in CO<sub>2</sub> incubator (5% CO<sub>2</sub>) (Thermo Scientifics, Germany) for three weeks. The medium was added every three days. After three weeks, colonies were stained with 0.5% crystal violet (Sigma, St. Louis, MO) and washed with PBS to remove excessive dye. Stained colonies were counted individually using inverted phase microscope (Zeiss, Axiovert 25, Germany).

## E. Live / Dead Assay

Live/Dead assay was used (Molecular Probes, Carlsbad, CA) to determine the methanolic extract of B. aristata cell apoptotic effects in MCF-7 cells. This method determines intracellular esterase activity and plasma membrane integrity. The assay measures the emitted fluorescence intensity by enzymatic conversion of cell permeable non-fluorescent calcein AM with ubiquitous intracellular esterase present in live cells. In addition, dead cells were quantitated with ethidium bromide, a red fluorescent homodimer dye which can enter dead cells through damaged membranes and bind to nucleic acids (Ali et al., 2008). Briefly 1×10<sup>4</sup> MCF-7 cells were incubated in 24 well culture plates either with B. aristata methanolic extract (125, 250 and 500 µg) for 48 h at 37 °C. After incubation, the cells were stained with Live/Dead kit agents for 30 min at 37 °C as per the manufacturer's instructions. The number of live and dead cells were observed under a fluorescence microscope (Olympus, Germany), followed by counting live (green at excitation and emission wavelengths of 495 and 515 nm, respectively) and dead (red at excitation and emission wavelengths of 495 and 635 nm, respectively) cells.

## F. In vitro Scratch Assay

MCF-7 ( $5 \times 10^4$ ) cells were seeded in 60 mm culture plates for monolayer formation up to 80% confluence and the cells were subjected to serum starvation for 2 h and treated for 48 h with methanolic extract of *B. aristata* (125 and 250 µg) for 48 h. The drug containing medium was removed, scratch was created by sterile p200 micropipette tip and 2% media was added after PBS washes at least twice to remove floating cells. Photographic images were taken at 0, 12, 24 and 48 h using Zeiss inverted phase microscope (Axiovert 25). Cell migration was expressed as the percentage of the gap relative to the total area of the cell-free region immediately after the scratch using ImageJ software (National Institutes of Health, Bethesda, MD, USA) (Tang *et al.*, 2013).

#### G. Statistical Analysis

Data were expressed as Mean  $\pm$  S.D obtained from three independent experiments. The data was analyzed by analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. The level of significance was set at  $p \le 0.05$ .

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III. RESULTS
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## A. Anti-Proliferative Effects of Methanolic Extract of B. Aristata in MCF-7 Cells

Anti-proliferative activity of methanolic extract of *B. aristata* in MCF-7 cells was determined by MTT assay as shown in Fig. 1. Methanolic extract of *B. aristata* exhibited a suppressive effect on cell proliferation in dose dependent manner.



Figure 1. Dose of B. aristata stem methanolic extract effect on viability of MCF-7 cells. Cell viability of untreated control was set as 100% to calculate Data were expressed as mean  $\pm$  SD (n = 3). \* $p \le 0.001$ .

Cell proliferation was significantly ( $p \le 0.001$ ) decreased by viabilities of the rest of the treated groups. 35% at 500 µg of methanolic extract of *B. aristata* for 48 h. The IC<sub>50</sub> value was determined based on viability rates of cells that treated with varying concentrations of the drug for 48 h. The IC<sub>50</sub> was 220 µg for methanolic extract of *B. aristata*.

#### B. Inhibition of Anchorage Independent Growth of MCF-7 Cells

To determine *in vitro* anti-neoplastic effect of methanolic extract of *B. aristata*, soft agar colony formation assay was performed in MCF-7 cells. The number of colonies in untreated cells and various treatment groups was counted and is summarized in Fig. 2. From these results, it is evident that treatment of 500 µg caused significant inhibition (80%) of colony formation which is consistent with the MTT assay ( $p \leq 0.001$ ).



Figure 2. Effect of B. aristata stem methanolic extract on colony formation (anchorage independent growth) of MCF-7 cells in soft agar. The percentage of colonies counted in plates was expressed as mean  $\pm$  SD (*n*=3). \**p*  $\leq$  0.005.

## C. B. aristata Enhanced the Apoptosis in MCF-7 Cells In Vitro

Further, the potentiating effect of methanolic extract of *B. aristata* induced apoptosis in MCF-7 cells was determined by using Live/Dead assay. MCF-7 cells were treated with methanolic extract of *B. aristata* were shown that significant ( $p \le 0.001$ ) increase of apoptotic cells, i.e., 53 and 67.5% at 250 and 500 µg respectively. On the contrary, lesser apoptosis was observed at 125 µg of methanolic extract (24%). These results indicated a methanolic extract of *B. aristata* had a shown significant effect on apoptosis in MCF-7 cell lines.



Figure 3. MCF-7 was treated with methanolic extract of B. aristata. Cell apoptosis was determined by Live/Dead assay. The results represent mean  $\pm$ SEM for triplicate experiments.

## D. Methanolic Extract of B. aristata Inhibits the MCF-7 Cell Migration

The effect of methanolic extract of *b. aristata* on cell migration was examined up to 48 h by *in vitro* scratch assay (Fig. 4). The MCF-7 cells were treated with various concentrations of methanolic extract of *B. aristata* (125 and 250 µg). In untreated cells, the cell migration observed at 48 h is 100%. Such a cell migration was inhibited by 16.2% in presence of 125 µg of methanolic extracts, where as the cell migration was significantly ( $p \le 0.001$ ) inhibited up to 50% when the cells were treated with 250 µg of methanolic extracts for 48 h.



Figure 4. Effect of methanolic extract of B. aristata on cell migration was monitored at 0, 12, 24 and 48 h and the cell migration was analyzed as described in 'Materials and Methods. Data were expressed as mean  $\pm$  SD (*n*=3).\**p* $\leq$  0.001.

# IV. DISCUSSION

In vitro cytotoxic activity against MCF-7 cell line at different concentrations of methanolic extracts of B. aristata was evaluated. Cytotoxic effect against the breast cancer cell line is considered as a prognostic anticancer activity indicator and IC50 value calculated for B. aristata methanolic extract is 220 µg (Fig. 1), which indicates potentially presence of cytotoxic activity and should be evaluated against primary cell lines to examine the selectivity of their effects. The exhibited cytotoxic activity in MCF-7 cell lines may be due to the presence of alkaloids in the methanolic extract of B. aristata stems was investigated earlier in photochemical which screening of the extract (Das et al., 2009). B. aristata has been used for medicinal applications traditionally in India and its therapeutic investigations needed to provide some additional insight for using as curative agent for breast cancer. Recently, anti-cancer properties of berberine, a major compound in this plant, that has shown the inhibition of cancer cell proliferation, metastasis, angiogenesis, activation of apoptosis, DNA binding and inhibition of telomerase (Malla and Kumari, 2013; Li et al., 2013). Previous studies revealed the anti-proliferation activity of berberine (Letasiova et al., 2005) through influencing the mitochondrial trans-membrane potential, metalloproteinase (MMP) regulation, matrix p53 activation, nucleated factor -kappa B (NF-kB) signal activation (Wen et al., 2011) and dose-dependent reduction in cancer cell growth assessed by increased DNA content in G2/M and S phases (Lin et al., 2006) and targeted AMP activated protein kinase, which regulates tumor progression and metastasis (Park et al., 2012, Wang et al., 2012). Further, the 48 h exposure of methanolic extracts of B. aristata to MCF-7 cell lines in present study exhibited a suppressive effect significantly  $(p \le 0.001)$  on anchorage dependent growth measured by soft agar assay (Fig. 2) and cell migration (Fig. 4) as well as increased apoptotic cells (Fig. 3). Such an antineoplastic and anti-metastatic effect of berberine and siRNA synergistically was previously reported in bladder cancer cells through attenuating the migration and invasion of bladder cancer cells (Yan et al., 2013). It also suppress tissue plasminogen activator induced PKC-a phosphorylation which leads to inhibition of MMP-1 and MMP-9 expression (Kim et al., 2012) and also via Akt / NK-kB and AP-1 signaling pathway (Kuo et al., 2012) in breast cancer cells. In addition, berberine exhibited p53dependent apoptotic death in human neuroblastoma and prostate cancer cells (Choi et al., 2009) and inhibition of telomerase activity in nasopharyngeal carcinoma cells (Biao et al., 2007). In present study the metholoic extracts of B. aristata resulted anticancer activity significantly in MCF-7 breast cancer cell lines, it may be postulated that the plant extract would be helpful in pharmacological applications in treatment of breast cancer. However, the present study would be helpful to investigate further in elucidating the mechanism involved by active components such as berberine in anticancer activity.

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