Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 5, 2014

Original Article

CYTOTOXIC ACTIVITY OF *BROUSSONETIA PAPYRIFERA*(L.) VENT ON MCF-7, HELA AND HEPG2 CELL LINES

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Received: 20 Mar 2014 Revised and Accepted: 20 Apr 2014

ABSTRACT

Objective: *Broussonetia papyrifera* is widely used in Chinese traditional medicine. The current study was carried out to evaluate cytotoxic activity of methanolic extracts of leaf, bark and fruit on MCF-7, HeLa and HepG2 cell lines.

Methods: The cytotoxic activity was determined as percentage of growth inhibition and cytotoxicity by Trypan blue dye exclusion assay and MTT assay respectively.

Results: Trypan blue dye exclusion assay of leaf extract showed potent cytotoxic activity on MCF-7 and HeLa cell lines with IC_{50} values 105 μgmL^{-1} and 110 μgmL^{-1}. The MTT assay confirmed the cytotoxicity of leaf extract with IC_{50} values 87.5 μgmL^{-1} and 106.2 μgmL^{-1} respectively. The bark extract showed better activity on HeLa cell line with IC_{50} 75.3 μgmL^{-1} and 88.3 μgmL^{-1}. The leaf and bark extracts exhibited moderate activity on HepG2 cell line. Methanolic extract of fruit indicated insignificant cytotoxic activity against three cell lines tested.

Conclusion: Further, detailed evaluation of leaf and bark extracts seems promising in formulation of anticancer drugs against cervical and breast cancer.

Keywords: *Broussonetia papyrifera*, Cytotoxicity, HeLa, HepG2, MCF-7.

INTRODUCTION

Cancer is a life threatening disease as treatment is difficult due to ineffective and expensive drugs coupled with numerous side effects. Efforts are being made for the identification of naturally occurring anticancer compounds that would be useful in treatment of cancer. Plants are regarded as reservoir of many compounds which are important in the treatment of cancer. Plant secondary metabolites, their semi-synthetic and synthetic derivatives are vital sources of anticancer drugs. It is estimated that more than 50 % of antitumor drugs which are under clinical trials, are from plants [1, 2]. Alkaloids such as vinblastine and vincristine isolated from *Catharanthus roseus* are well known examples of plant derived anticancer agents [3, 4].

Broussonetia papyrifera (L.) Vent, belonging to Moraceae commonly known as Paper mulberry, is widely distributed in temperate and tropical regions. It is native to Eastern Asia and is distributed throughout China, Korea, Thailand and Japan. The plant is highly invasive and commercially important as the stem bark is used in paper making. The plant has significant medical applications in traditional Chinese medicine in view of its astringent, diuretic, vulnerary, tonic, ophthalmic, diaphoretic and laxative properties [5-7]. Compounds such as phenols, flavonoids and alkaloids isolated from various parts of *B. papyrifera* have shown significant anti-inflammatory, antinociceptive, anti-hepatotoxic, antimicrobial and antioxidant activities [8-13]. The plant is also known to possess aromatase, tyrosinase, α-glucosidase and PTP1B inhibitory activities [10, 13-16].

Considering the medicinal uses and applications of *B. papyrifera*, the present study focuses on evaluating cytotoxic activity of methanolic extracts of leaf, bark and fruit on HeLa, MCF-7 and HepG2 cell lines.

MATERIALS AND METHODS

Plant material

The plant samples were collected from Bangalore city and identified as *Broussonetia papyrifera* (L.) Vent. using Flora of Bangalore [17]. Authorized authentication was done by National Ayurvedic Dietetics Research Institute, Bangalore; vide voucher specimen number RRCBI/MCW/09. Separate voucher specimen (BP01) is maintained in the herbarium of the research centre.

Preparation of methanolic extracts

The leaves, bark and fruits were air dried and powdered, exhaustively extracted with methanol using soxhlet apparatus and extracts were concentrated to dryness under reduced pressure using vacuum rotary evaporator. The dried methanolic extracts were stored at 4 ˚C and used for cytotoxic studies.

Chemicals

Dulbecco's Modified Eagle Medium (DMEM), Fetal Bovine Serum (FBS), Phosphate Buffered Saline (PBS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), Trypan blue and Trypsin were purchased from Sigma-Aldrich Corporation, St. Louis, USA. Antibiotics, EDTA and Glucose from HiMedia Laboratories Pvt. Ltd., Mumbai, India and Dimethyl Sulfoxide (DMSO) and Propanol were obtained from Merck Limited, Mumbai, India. All other chemicals and reagents used were analytical grade.

Cell lines and Culture medium

MCF-7 (Human breast adenocarcinoma cell line), HeLa (Human epithelial carcinoma cell line) and HepG2 (Human hepatocellular liver carcinoma cell line) were procured from National Centre for Cell Sciences (NCCS), Pune, India. The cell lines were cultured in DMEM supplemented with 10 % inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/mL), streptomycin (100 μgmL^{-1}) and amphotericin B (5 μgmL^{-1}) in a humidified atmosphere of 5 % CO_2 at 37 ˚C. The cells were dissociated with TPVG (0.2 % trypsin, 0.02 % EDTA, 0.05 % glucose in PBS). The stock cultures were grown in 25 cm^3 culture flasks and all experiments were carried out in 96 well microtiter plate (Tarsons India Pvt. Ltd., Kolkata, India).

Preparation of test solutions

For cytotoxicity studies, each weighed test drug was separately dissolved in distilled DMSO and the volume was made up with DMEM supplemented with 2 % inactivated FBS to obtain a stock

solution of 1 µgmL^{-1} concentration and sterilized by filtration. Serial two fold dilutions were prepared from stock for carrying out cytotoxic studies.

Trypan blue dye exclusion method

The dye exclusion test is used to determine the number of viable cells present in a cell suspension. The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/mL using DMEM containing 10 % FBS. To each of 40 mm petridish, 1 mL of the diluted cell suspension was added. After 24 hours, a partial monolayer was formed. After removing the supernatant the monolayer was washed once with medium. Different concentrations of test drug were prepared and 1 mL of each concentration was added on to the partial monolayer in separate culture dishes. The culture plates were then incubated at 37 ˚C for 3 days in 5 % CO_2 atmosphere and microscopic examination was carried out at every 24 hours. After 72 hours, the drug solutions in the wells were removed and cells were trypsinized. The cells were suspended in PBS and centrifuged to separate cell pellet, resuspended in 1 mL of fresh medium. Dye exclusion test was performed by mixing equal quantity of the drug treated cells and trypan blue (0.4 %) and left for a minute. The cell suspension was loaded in a hemocytometer [18]. Viable and non-viable cell count was recorded within two minutes. The percentage of growth inhibition was calculated and IC_{50} value was calculated from the dose-response curves for each cell line.

$$\text{Growth inhibition (\%)} = \frac{\text{Non-viable cells}}{\text{Total cells}} \times 100$$

MTT assay

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/ mL using DMEM containing 10 % FBS. To each well in the 96 well microtiter plate, 0.1 mL of the diluted cell suspension was added. After 24 hours, a partial monolayer was formed and the supernatant was drained off. The monolayer culture was washed with medium and 100 µL of different concentrations of test drugs were added on to the partial monolayer in microtiter plate. Cultures were incubated at 37 ˚C for 3 days in 5 % CO_2 atmosphere and at every 24 hour interval, cultures were examined microscopically and observations were recorded. After 72 hours incubation, the drug solutions in the wells were discarded and 50 µL of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 hours at 37 ˚C in 5 % CO_2 atmosphere. Supernatant was removed and 100 µL of propanol was added and the plates were gently shaken to solubilize the formazan and absorbance was measured at 540 nm [19]. The relative growth inhibition (%) was expressed as a percentage relative to the untreated control cells.

$$\text{Cytotoxicity (\%)} = \frac{\text{(Absorbance of control - Absorbance of test group)}}{\text{Absorbance of control}} \times 100$$

Concentration of test drug needed to inhibit cell growth by 50 % (IC_{50}) was generated from the dose-responsive curves for each cell line.

Statistical analysis

The experimental results were expressed as mean ± SD. The significance of difference among test groups and control group were determined by means of one-way ANOVA. The level of significance was set at $p \leq 0.05$. IC_{50} values were calculated using linear regression method of plots of the cell inhibition percentage against the concentration of tested sample using Microsoft Excel software.

RESULT AND DISCUSSION

Plant derived drugs have been extensively screened for anticancerous property. In this study *B. papyrifera* was evaluated for cytotoxic activity against MCF-7, HeLa and HepG2 cell lines by Trypan blue dye exclusion method and MTT assay. The IC_{50} values for methanolic extracts of leaf, bark and fruit against three cell lines are listed in Table 1. A decrease in the cell count was observed with increase in extract concentration (Fig. 1-6). There was a dose dependent increase in the cytotoxic activity for all the concentrations tested.

Table 1: Cytotoxic activity (IC_{50}) of methanolic extracts of *B. papyrifera*

Sample	Trypan blue dye exclusion method IC_{50} (µgmL^{-1})			MTT assay IC_{50} (µgmL^{-1})		
	MCF-7	HeLa	HepG2	MCF-7	HeLa	HepG2
Leaf	105±0.31*	110±0.76*	415±0.23*	87.5±0.86*	106.2±0.09*	325±0.57*
Bark	122.3±0.33*	75.3±0.59*	281.3±0.46*	130±0.07*	88.3±0.39*	192±0.14*
Fruit	590±0.26*	>1000	700±0.41*	550±0.45*	470±0.93*	670±0.02*

n=18, *p ≤ 0.05

The leaf extract exhibited potent cytotoxic activity against MCF-7 with IC_{50} values 105 µgmL^{-1} and 87.5 µgmL^{-1} obtained by Trypan blue dye exclusion method and MTT assay respectively. It showed slightly lesser cytotoxicity against HeLa cell line when compared to MCF-7 cell line. Although compounds which are cytotoxic to HepG2 have been isolated from leaves [20], the present study revealed that crude methanolic extract of *B. papyrifera* leaves has moderate activity against HepG2 cell line.

Bark extract showed more cytotoxicity on HeLa cells with IC_{50} values 75.3 µgmL^{-1} and 88.3 µgmL^{-1} followed by MCF-7 cell line with 122.3 µgmL^{-1} and 130 µgmL^{-1} obtained from Trypan blue dye exclusion method and MTT assay respectively. Cytotoxic activity of dichloromethane and butanol fractions from stem bark has been determined against HT-29, human colon cancer cell line [21]. Broussoflavonol B, a chemical purified from bark restricted the growth of ER- negative breast cancer stem-like cells, SK-BR-3 cells [22] and MDA-MB-231 cells [23]. In support of earlier studies, methanolic extract of bark was found to possess potent cytotoxicity against MCF-7, a human breast adenocarcinoma cell line. Three compounds, kazinol B, broussochalcone A and papyriflavonol A isolated from root bark were tested against HepG2 cell line and the latter one was found to be a potent anticancer compound [11]. However in this study the crude methanolic extract of stem bark was found to have moderate activity against HepG2 cell line.

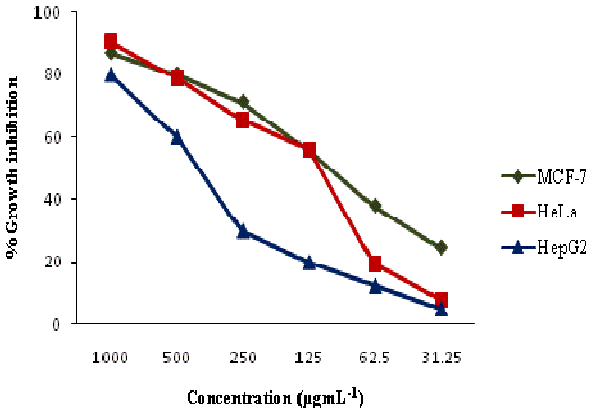

Fig. 1: Cytotoxic activity of leaf extract by Trypan blue dye exclusion method

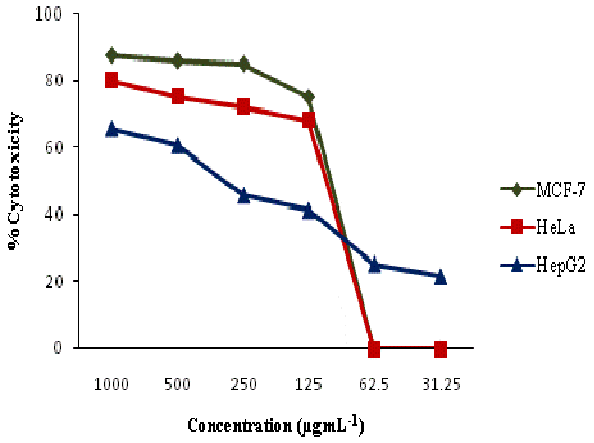

Fig. 2: Cytotoxic activity of leaf extract by MTT assay

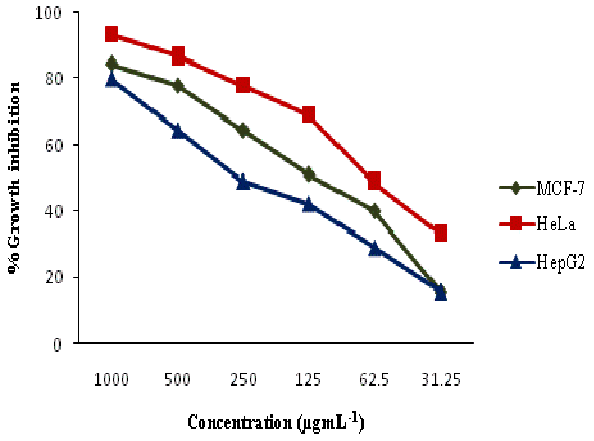

Fig. 3: Cytotoxic activity of bark extract by Trypan blue dye exclusion method

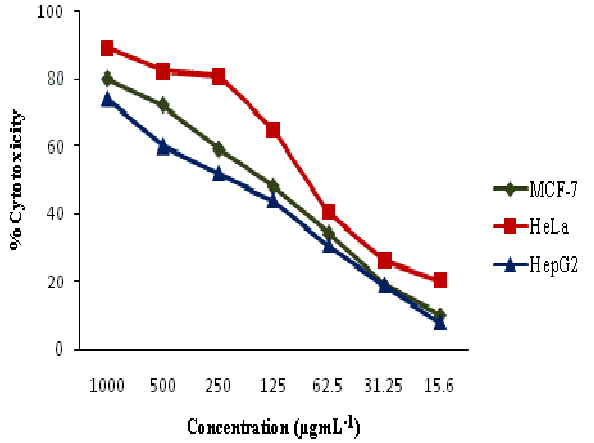

Fig. 4: Cytotoxic activity of bark extract by MTT assay

The methanolic extract of fruit showed insignificant cytotoxicity against MCF-7, HeLa and HepG2 cell lines. It is the first instance where the *B. papyrifera* fruits have been evaluated for cytotoxic activity.

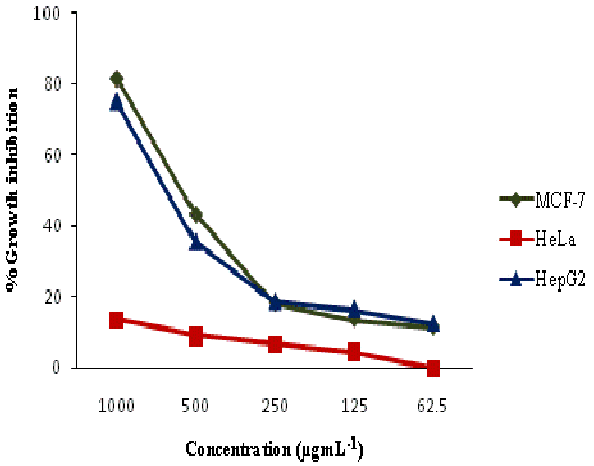

Fig. 5: Cytotoxic activity of fruit extract by Trypan blue dye exclusion method

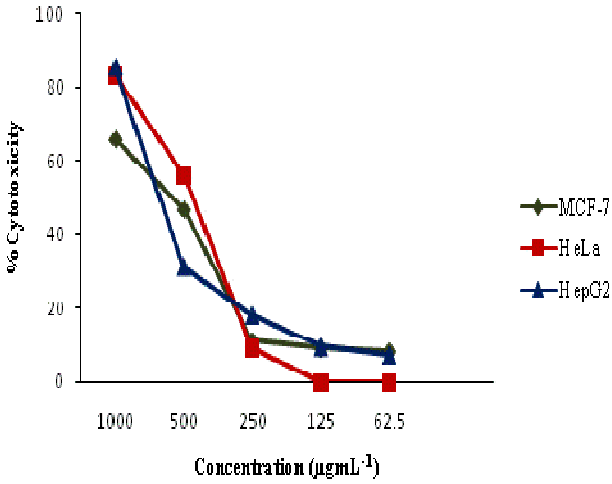

Fig. 6: Cytotoxic activity of fruit extract by MTT assay

CONCLUSION

The leaf and bark extracts of *B. papyrifera* showed potent cytotoxic activity against MCF-7 and HeLa cell lines. Therefore isolation and characterization of compounds responsible for the cytotoxic activity and their evaluation as anticancer agents against human breast and cervical cancer is necessary.

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