BJP

Bangladesh Journal of Pharmacology

Research Article

Anti-cancer activity of *Morus nigra* leaves extract

A Journal of the Bangladesh Pharmacological Society (BDPS); www.bdps.info
Journal homepage: www.banglajol.info
Abstracted/indexed in Academic Search Complete, Agroforestry Abstracts, Asia Journals Online, Bangladesh Journals Online, Biological Abstracts, BIOSIS Previews, CAB Abstracts, Current Abstracts, Directory of Open Access Journals, EMBASE/Excerpta Medica, Google Scholar, HINARI (WHO), International Pharmaceutical Abstracts, Open J-gate, Science Citation Index Expanded, SCOPUS and Social Sciences Citation Index
Bangladesh J Pharmacol 2014; 9: 496-97
ISSN: 1991-0088

Anti-cancer activity of *Morus nigra* leaves extract

Muhammad Imran Qadir[1,2], Muhammad Ali[1] and Zubair Ibrahim[2]

[1]Institute of Molecular Biology & Biotechnology, Bahauddin Zakariya University, Multan, Pakistan; [2]College of Pharmacy, GC University, Faisalabad, Pakistan.

Article Info

Received: 8 August 2014
Accepted: 31 August 2014
Available Online: 9 October 2014

DOI: 10.3329/bjp.v9i4.19783

Cite this article:
Qadir MI, Ali M, Ibrahim Z. Anti-cancer activity of *Morus nigra* leaves extract. Bangladesh J Pharmacol. 2014; 9: 496-97.

Abstract

Plants are screened for treatment of many ailments including cancer because they possess certain potential constituents which are effective for treatment. The aim of this study was to evaluate the anti-cancer activity of *Morus nigra* leaves against human cervical cancer cell line (HeLa). *n*-Hexane and aqueous methanolic extract of plant's leaves were made by maceration. Anti-cancer activity was estimated by methyl-thiazolyl-tetrazolium (MTT) assay and percentage inhibition of cells was calculated. Results of MTT showed that 100 µg/mL aqueous methanol extract of *M. nigra* inhibited 89.5 - 32.0% of HeLa cell line. It was concluded that *M. nigra* possess anti-cancer activity.

Introduction

Cancer is one of the leading death causing disease of the current era and is characterized by aberrant growth of cell mass that is uncoordinated to remain proliferating when stimulus has been removed that provoked that action. Many plants have recently be reported with anticancer activity like *Casuarina equisetifolia* (Shafiq et al., 2014), *Aspergillus niger* (Channabasavaet al., 2014) and *Convolvulus arvensis* (Saleem et al., 2014).

M. nigra (Family Moraceae), commonly as Black Mulberry (English) and Shah-toot (Hindi/Urdu), is used as hepatoprotective (Malhi et al., 2014), anti-oxidant (Imran et al,. 2010; Ercisli and Orhan, 2008) and antimicrobial (Digrak et al., 1999).

The objective of this study was to evaluate the anti-cancer activity of *M. nigra* extracts against human cervical cancer cell line ((HeLa).

Material and Method

Collection of medicinal plants

M. nigra leaves weres collected from Jinnah colony, plant nurseries located at Bilal road and Samundri road, Faisalabad. The plant material was identified from Department of Botany, University of Agriculture, Faisalabad. Prior to maceration, all plants were washed, shadow dried and grinded to coarse powder for extract formation.

Preparation of extracts

One thousand three hundred grams of powdered *M. nigra* (leaves) was merged in 4,000 mL of *n*-hexane and aqueous methanol (70% methanol and 30% distilled water) for 7 days with occasional shaking. After maceration, rotary evaporator lyophilizer was used for concentration of the extracts.

Anti-cancer activity

Single cell suspension was made by trypsin-ethylene-diamine tetraacetic acid (EDTA) and layer of cells was separated. Final density of 1 x 10^5 was made using medium containing 5% FBS and diluted the cell suspension. In 96-well plates, 10,000 cells/well were seeded and incubated at 37°C, keeping 5% CO_2, 95% air and 100% relative humidity. After 24 hours, different concentrations of extracts i.e., 1, 10, 25, 50 and 100 µg/mL were added and incubated under above mentioned working conditions for another 48 hours. Well without plant extracts were taken as control. Whole procedure was repeated in triplicate and viable cells were calculated using hemocytometer before and after

extracts addition (Dantuet al., 2012).

MTT assay

In every well 100 µL of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) in phosphate buffered saline was added and incubated at 37°C for 4 hours. The medium with MTT was flipped off and the formed formazan crystals was solubilized in 100 µL of DMSO. Using micro plate reader the absorbance was measured at 490 nm. The %cell inhibition was determined using the following formula (Cheng et al., 2011).

$$\%\text{Cell inhibition} = \frac{1 - \text{Absorbance sample}}{\text{Absorbance control}} \times 100$$

Graph was plotted against concentrations to calculate IC_{50}.

Statistical analysis

A logistic linear regression model was fit to the data using Microsoft Excel 2013 Software to calculate the IC_{50}. The data obtained were expressed as mean ± standard deviation. A value of $p<0.05$ was considered as significant.

Results and Discussion

The anti-cancer activity of *n*-hexane and aqueous metha -nolic extract of *M. nigra* (leaves) against HeLa cancer cell line is shown in Table I. 100 µg/mL aqueous methanol extract of *M. nigra* inhibited 89.5-32.0% of HeLa cell line. Estimated IC_{50} of *n*-hexane and aqueous methanolic of *M. nigra* against HeLa cancer cell line at 24 hours was 185.9 ± 8.3 and 56.0 ± 1.7 µg/mL respectively. *n*-Hexane and aqueous methanolic extract at 1, 10, 25, 50 and 100 µg/mL had shown dose dependent inhibition of cells.

Sundararajan et al. (2006) also evaluated anti-cancer activity of *n*-hexane extract of *Bidens pilosa* on HeLa and KB cell lines corroborated our findings with IC_{50} values of 509.2 ± 6.3 and 385.2 ± 4.7 µg/mL respectively. A lower IC_{50} of *Carthamus oxyacanthus* (whole plant) indicates that it has phytochemical constituents that synergistically inhibit growth of cancer cells (Alesiani et al., 2010).

Table I

Percentage inhibition (Mean ± SD) of HeLa cell line by *Morus nigra* extracts

Concentration (µg/mL)	*n*-Hexane	Aqueous methanol
1	0.04 ± 0.0	0.8 ± 0.1
10	4.5 ± 0.8	7.2 ± 1.1
25	17.9 ± 0.5	20.8 ± 2.2
50	30.9 ± 0.1	48.1 ± 1.3
100	41.2 ± 0.8	89.5 ± 2.0
IC_{50}	185.9 ± 8.3	56.0 ± 1.6

It can be concluded that *M. nigra* (leaves) possess anti-cancer activity against cervical (HeLa) cancer cell line.

Reference

Alesiani D, Canini A, D'Abrosca B, DellaGreca M, Fiorentino A, Mastellone C, Pacifico S. Anti-oxidant and anti-proliferative activities of phytochemicals from Quince *Cydonia vulgaris* peels. Food Chem. 2010; 118: 199-207.

Channabasava, Govindappa M. First report of anti-cancer agent, lapachol producing endophyte, *Aspergillus niger* of *Tabebuia argentea* and its *in vitro* cytotoxicity assays. Bangladesh J Pharmacol. 2014; 9: 129-39.

Cheng XL, Liu Q, Peng YB, Qi LW, Li P. Steamed ginger (*Zingiber officinale*): Changed chemical profile and increased anticancer potential. Food Chem. 2011; 129: 1785-92.

Dantu AS, Shankarguru P, Ramya DD, Vedha HB. Evaluation of *in vitro* anti-cancer activity of hydroalcoholic extract of *Tabernaemontana divaricata*. Asian J Pharm Clin Res. 2012; 5: 59-61.

Digrak M, Alma MH, İlçim A, Sen S. Antibacterial and antifungal effects of various commercial plant extracts. Pharm Bio. 1999; 37: 216-20.

Ercisli S, Orhan E. Some physicochemical characteristics of black mulberry (*Morusnigra* L.) genotypes from northeast Anatolia region of Turkey. Scientia Horticulturae. 2008; 116: 41-46.

Imran M, Khan H, Shah M, Khan R, Khan F. Chemical composition and anti-oxidant activity of certain *Morus* species. J Zhejiang Uni Sci B. 2010; 11: 973-80.

Imran M, Talpur FN, Jan MI, Khan A, Khan I. Analysis of nutritional components of some wild edible plants. JCSP. 2007: 29: 500-08.

Mallhi TH, Qadir MI, Khan YH, Ali M. Hepatoprotective activity of aqueous methanolic extract of *Morus nigra* against paracetamol-induced hepatotoxicity in mice. Bangladesh J Pharmacol. 2014; 9: 60-66.

Saleem M, Qadir MI, Ahmad B, Saleem U, Naseer F, Schini-Kerth V, Ahmad M, Hussain K. Cytotoxic effect of ethanol extract of *Convolvulus arvensis* L (Convolvulaceae) on lymphoblastic leukemia Jurkat cells. Trop J Pharm Res. 2014; 13: 705-09.

Shafiq Y, Naqvi BS, Rizwani GH, Usman M, Shah BA, Aslam M, Hina B. Anti-acne activity of *Casuarina equisetifolia* bark extract: A randomized clinical trial. Bangladesh J Pharmacol. 2014; 9: 337-41.

Sundararajan P, Dey A, Smith A, Doss AG, Rajappan M, Natarajan S. Studies of anti-cancer and antipyretic activity of *Bidens pilosa* whole plant. Afric Health Sci. 2006; 6: 27-30.

Author Info
Muhammad Imran Qadir (Principal contact)
e-mail: mrimranqadir@hotmail.com