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 been reported with anticancer activity like *Casuarina equisetifolia* (Shafiq et al., 2014), *Aspergillus niger* (Channabasava et 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), antioxidant (Imran et al., 2010; Ercisli and Orhan, 2008) and antimicrobial (Digbak et al., 1999).

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

Material and Method

Collection of medicinal plants: *M. nigra* leaves were 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.

Anticancer activity: Single cell suspension was made by trypsin-ethylenediamine 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 (Dantu et 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).

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

Graph was plotted against concentrations to calculate IC₅₀.

**Statistical analysis:** A logistic linear regression model was fit to the data using Microsoft Excel 2013 Software to calculate the IC₅₀. The data obtained were expressed as mean ± standard deviation. A value of p<0.05 was considered as significant.

**Results and Discussion**

The anticancer activity of n-hexane and aqueous methanolic 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 IC₅₀ of n-hexane and aqueous methanolic of *M. nigra* against HeLa cancer cell line at 24 hours was 185.9 ± 8.3 µg/mL 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 anticancer activity of n-hexane extract of *Bidens pilosa* on HeLa and KB cell lines corroborated our findings with IC₅₀ values of 509.2 ± 6.3 µg/mL and 385.2 ± 4.7 µg/mL respectively. A lower IC₅₀ of *Carthamus oxyacanthus* (whole plant) indicates that it has phytochemical constituents that synergistically inhibit growth of cancer cells (Alesiani et al., 2010).

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

**Reference**


| 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 |
| IC₅₀ | 185.9 ± 8.3 | 56.0 ± 1.6 |