Research Article

Genotoxicity Potential of *Bacopa monnieri* on Oral Cancer Cell Lines by DNA Fragmentation

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Accepted on: 18-05-2016; **Finalized on:** 30-06-2016.

ABSTRACT

The aim of the study is to determine the genotoxicity analysis of *Bacopa monnieri* on oral cancer cell lines by DNA fragmentation method. This study was done to evaluate the anti-cancer activity of *Bacopa monnieri* extract. *Bacopa monnieri* is a perennial, creeping and non-aromatic herb native to the wetlands of Southern India, Australia, Europe, Africa, Asia, North and South America. It is also known as Brahmi. It displays in vitro antioxidant and cell-protective effects. In animals, it also inhibits acetylcholinesterase, activates choline acetyltransferase, and increases cerebral blood flow. It is used in traditional Ayurvedic treatment for epilepsy, asthma, ulcers, tumors, ascites, enlarged spleen, anaemia, inflammations, leprosy and gastroenteritis. This study may help in the formulation of economical and new anticancer agents derived from *Bacopa monnieri.* The *Bacopa monnieri* extract showed effective anticancer activity against oral cancer cell lines.

Keywords: monnieri, bacopa, lines, oral, cancer, cell, anticancer, activity, extract.

INTRODUCTION

Cancer is an important public health problem in most parts of the world and oral cancer is among the 10 most common cancers in the world. Squamous cell carcinoma is the most common malignant neoplasm in the oral cavity. These malignancies are often not examined until a late stage. The risk factors associated with it's occurrence are tobacco and heavy alcohol consumption[1]. Almost eighty three percent of the oral cancer patients use tobacco and the rest seventeen percent are non-users. The mean age of tobacco users is 51 years and of non-users was 52 years[2].

So, it mostly occurs in middle aged and older individuals. Women have been more equally exposed to known oral carcinogens such as tobacco and alcohol[3].

Baccopa monnieri is a miniature and a creeping herb usually known by the name "Brahmi". It belongs to the Scrophulariaceae family. It has been approved by several ancient Ayurvedic treatises for the enhancement of memory capacity and remedy of mental disorders. It's extract or bacosides have also shown anxiolytic effect, anti-depressant activity and anti-convulsive action[4]. It helps to repair damaged neurons in selective places of the brain[5]. This medicinal plant is analyzed phytochemically to have a chalcone. Indian Ayurvedic system suggests that this herb is useful as emetic, laxative, and as medicine in treating bad ulcers, tumors, enlargement of spleen, indigestion, inflammation and anemia. It is reported to improve the intellect and is used as medicine for the treatment of respiratory disease like asthma, hoarseness, insanity and as a potent nerve tonic, cardio tonic, and diuretic.[6]

The sap of the leaves is given to children for relieving bronchitis and diarrhoea. Rheumatism condition can be treated by the remedy obtained from the paste of the leaves[7]. India holds a well recorded and traditionally well practiced knowledge of herbal medicine.

Although more than 1500 anticancer drugs are in active development with over 500 of the drugs under clinical trials, there is an urgent need to develop much effective and less toxic drugs[8].

The various contents in the herb act on different anticancer pathways. These contents might be selective for carcinogenic cells instead of normal cells[9]. The pharmacological mechanisms of most natural anticancer compounds remain incomprehensible, which has become one of the major obstacles in the development of new and effective anticancer agents[10].

Three quarters of anti-cancerous compounds used in medicine are natural products or related to them. Of the 140 anti-cancer agents approved since 1940 and available for use, over 60% can be traced to a natural product. Of the 126 small molecules among them, 67% are natural in origin[11].

MATERIALS AND METHODS

The chemicals and reagents used were purchased from Himedia.

Preparation of Plant Extract

The herb *Bacopa monnieri* has active components like glycosides and henceforth, a polar solvent like aqueous ethanol is used.

The samples of the plant (air dried and powdered) was bought commercially. The dried powder was cold

extracted with 50% ethanol by intermittent stirring. This process was repeated twice[12]. The pooled extracts were concentrated, evaporated to dryness under reduced pressure. The extract was re-suspended in 1% gum acacia and subjected to various studies.

Maintenance of Cell Line

The vial containing KB cell lines acquired from ATCC was removed from liquid nitrogen freezer and the vial was thawed for 2 minutes by mild agitation in a 37°C water bath. Then, it was centrifuged for 10 minutes at 150 to 200 x g, room temperature. Supernatant was disposed and cells were cleansed with Eagle's minimum essential medium to remove residual DMSO. The cell pellet was re-suspended in 3ml of Dulbecco's Modified Eagle's Medium with 10% fetal bovine serum. It was then incubated in a CO_2 incubator. The culture was then kept in a growth medium with 10% FBS, until cell lines were re-established[13].

Isolation of DNA

$1*10^6$ cells were incubated with 100µl of cell lysis buffer at room temperature for an hour. This was centrifuged for 15 min at 3000rpm at 4°C to sediment the cell debris. To the supernatant equal volume of phenol: chloroform: isoamylalcohol mixture was added to the supernatant and mixed well.

This was centrifuged at 5000rpm for 15 min. The supernatant was transferred to new tube. And the centrifugation was repeated again.

To the final aqueous phase 40 µl of 3.5M ammonium acetate was added, to this ice cold isopropanol was added to precipitate the DNA. This was incubated at 20°C for 1 hour, followed by the centrifugation at 10000 rpm for 15min. The pellet was retained and washed with 70% ethanol and stored in 20-50 µl of TE buffer.

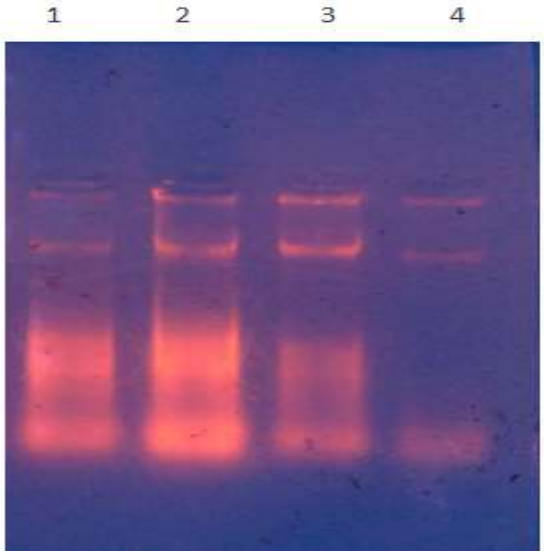
The samples were analyzed in 2% agarose gel stained with Ethidium bromide[14].

Genotoxicity Analysis by Agarose Gel Electrophoresis

Electrophoretic separation is usually carried out in agarose gels for most of the DNA samples. This is because DNA molecules and their fragments are larger than proteins; therefore larger size agarose gels are required. Any DNA fragment should move towards the anode with the same mobility under the influence of an electric field. This is due to the charge per unit length owing to the phosphate groups. Separation on agarose gels is achieved because of resistance to their movement caused by gel matrix. Thus the largest molecules will have difficulty moving, whereas the smallest molecules will be relatively unhindered. Gel concentrations must be chosen based on the molecules to be separated such as for plasmid molecules – 1% genomic DNA – 0.8% and RNA – 1.5%, mitochondrial DNA -0.8% and amplified samples at 1.5% was used.

The agarose gel has to be prepared with 1X TAE buffer and stained with 2µl of ethidium bromide. The % of agarose depends upon the molecule to be separated. Samples loaded with loading dye (2 µl of loading dye is used). Electrophoresis of DNA fragments at 50 volts. Visualization of DNA fragments in the UV trans-illuminator[14].

RESULTS AND DISCUSSION



Lane 1 - DNA from KB cells treated with 100µg sample

Lane 2 – DNA from KB cells treated with 200µg sample

Lane 3 – DNA from KB cells treated with 300µg sample

Lane 4 – DNA from untreated KB Cells

In diseases such as cancer, induction of apoptosis has been a new target for mechanism-based drug discovery. Apoptosis involves characteristic morphological and biochemical events ultimately leading to cell demise. *Bacopa monnieri* inhibits HeLa cell proliferation and accumulation of ascites. Induction of apoptosis on KB cells by *Bacopa monnieri* extract was validated by DNA fragmentation analysis using Agarose gel electrophoresis technique[15,16].

CONCLUSION

The chemotherapeutic agents long used in oncologic treatment produce detrimental side effects that amplify the mortality and morbidity caused by cancer. Henceforth, safer treatments are required.

The anticancer activity of the medicinal herbs selectively targets KB cells without affecting the normal cells and inhibits their growth. From the present study, it was concluded that the extract of *Bacopa monnieri* acts against oral cancer (KB) cells which may be due to the syngerstic effect of the secondary metabolites such as flavonoids present in the extract.

Thus, the anticancer activity of *Bacopa monnieri* may be useful in the treatment of patients with oral carcinoma[17].

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Source of Support: Nil, **Conflict of Interest:** None.