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Research Article

CYTOTOXICITY OF *PUNARNAVA* (*BOERHAAVIA DIFFUSA* L.) IN BREAST CELL LINE

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ABSTRACT

The incidence of cancer especially breast cancer is increasing alarmingly worldwide with a high percentage of death especially in developing countries. The Ayurvedic system treasures a host of medicinal formulations that have been shown to possess cytotoxic effects on tumor cell lines. Recently herbal medicines are coming to play a more vital role in the reduction and prevention of cancer. *Boerhaavia diffusa* L. (*Punarnava*), an annual herb has been used for managing wide range of diseases including cancer. This herb was also screened for various pharmacological activities like anti-inflammatory, anti-oxidant, immunomodulatory, anti-angiogenic, anti-metastatic activities and others. The decoction of root of *Boerhaavia diffusa* L. (*Punarnava*) was not scientifically evaluated for cytotoxicity. So the current study investigates the In vitro Cytotoxicity of root of decoction of *Punarnava* (*Boerhaavia diffusa* L.) in breast cell line (MCF-7). The five different concentrations of decoction of *Punarnava* (*Boerhaavia diffusa* Linn.) were used for Invitro Cytotoxicity by MTT assay at 24 hours and 48 hours. The test sample exhibits cytotoxicity of about 65.1±1.2at 800 µg/ ml concentration (48 hours) of incubation in MCF-7 breast cell line. The results were also analyzed statistically. It showed that there is highly significant difference in the percentage of inhibition of test sample in concentration from 50µg/ml -800µg/ml. The findings of this investigation concluded that the study drug *Boerhaavia diffusa* L. (*Punarnava*) has anti-cancer activity in MCF-7 breast cell line.

KEYWORDS: *Punarnava*, *Boerhaavia diffusa* L., Cytotoxicity, Breast cancer, Breast cell line, MTT assay, *Arbudam*.

INTRODUCTION

Cancer is one of the most dreadful diseases. Many efforts have been taken to find a complete cure but yet success is still far. Recent study showed that 7lakh people die of cancer every year in India. In 2012, WHO released new statistics on cancer incidence, mortality and prevalence worldwide (GLOBACAN 2012) estimates 28types of cancer in 184 countries in which there is more prevalence of breast cancer. [1]

On analyzing the description of neoplasm as *Granthi*, *Arbudam* etc in Ayurvedic classics, it can be assumed that *Arbudam* can be correlated as a malignant neoplasm[2]. Surgery, chemotherapy and radiotherapy are considered as the most common methods of cancer treatment. Although these methods are highly effective methods of cancer treatment, they exert severe side effects in use. One of the main problems in cancer treatment is gradual resistance of cancer cells against treatment. Therefore modern medical research focuses on finding new anticancer agents in order to reduce the existing resistance mechanisms. Herbs and other natural plant products have become the main source for this purpose. Herbal medicines play a vital role in the prevention and treatment of cancer. Herbal drugs include plants, herbal complexes or even a combination of plants, which were used thousand years before inventing modern drugs. The Ayurvedic system treasures a host of medicinal formulations that have been shown to possess cytotoxic effects on tumor cell lines. Several medicinal plants have been screened based on the integrative approaches on drug development from Ayurveda.

Boerhaavia diffusa L. from the family *Nyctaginaceae*, is a variable diffusely branched low spreading or creeping herbaceous perennial hogweed, commonly occurring abundantly in waste places, ditches and marshy places during rains[3] (Fig.1). It is a commonly used medicinal herb in Ayurveda. Root is mainly used in the medicinal preparations while the leaf is used as a food. Etymologically the term *Punarnava* (*punar* -again and

nava -fresh) that which becomes fresh again, that is it sprouts up or receives again every year [4]. The Ayurveda textbooks, which have evolved from the practical knowledge of the Vaidyas of Kerala prominently, mention widespread utility of this drug.

Qualitative analysis of root of *Punarnava* (*Boerhaavia diffusa Linn*) revealed that it contains an alkaloid Punarnavine which exhibits anti-inflammatory, anti-oxidant, immunomodulatory, anti-angiogenic, anti-metastatic activities. It induces apoptosis in B16F-10 melanoma cells. Roots are rich in rotenoids which is having anti-cancer activity is also proven. Boeravinone, which is also present in *Punarnava*, also shows anti-oxidant and anti-cancer activity. Ursolic acid is also proven that it inhibits various cancer cell types such as fibrosarcoma. It also induces apoptosis in certain cancer cells are also proven. In a test on oestrogen-responsive breast cancer cells (MCF-7) at doses of 20-320 mcg/ml, methanol extract of root of *Boerhaavia diffusa* reduced viability of cancer cells by 46.8%. Two rotenoids, boeravinones G and H isolated from roots were found to inhibit breast cancer resistance protein ABCG2 [5-10]. These findings suggest that the herb have the potential for the treatment of breast cancer. Hence the present study aimed to screen the cytotoxicity of concentrated decoction of *Boerhaavia diffusa* L. in breast cancer cell line (MCF 7).

MATERIALS AND METHODS

Materials

The plant material used in this research was mature root of *Punarnava* (*Boerhaavia diffusa* L.), collected from natural habitat and was shade dried and stored in airtight containers. (Fig.2) (Fig.3)

MCF-7 (Human Breast Cancer cells) was obtained from National Centre for Cell Sciences, Pune, India.

Methodology of preparation of drug

Step 1 (Preparation of *decoction*)

48gm of coarsely powdered root of *Punarnava* was mixed with 16 times of water and reduced to 96ml (1/8th) and strained through clean white cloth according to *Kwatha* preparation procedure mentioned in *Sarngadhara samhitha*.[11]

Step 2 (Filtration of decoction)

Using Whatman's filter paper of pore size 1 again decoction was filtered.

Step 3 (Concentration of Decoction)

Punarnava decoction was collected after the filtration. It was then transferred to borosil glass beaker and kept over a hot water bath and heated. Heating was continued till almost all water gets evaporated from the decoction. It was then stored in petridish and sealed it carefully and stored in refrigerator. Of them 0.002gm of concentrated decoction of *Punarnava (Boerhaavia diffusa* Linn) was dissolved in 1ml triple distilled water and was taken up for the study. (Fig.4) (Fig.5)

- **Tissue culture**

a. Sterilisation of glassware

All glassware and filtration apparatus used for tissue culture were soaked in solution of 5% Savlon overnight, cleaned using brush and washed thoroughly under running water. They were then soaked in boiling water for 15 minutes and rinsed in distilled water and dried in a hot air oven. These were then autoclaved at a pressure of about 15lbs for 15 minutes, dried and used for experiments.

b. Preparation of culture media

DMEM (Dulbecos Modified Eagle's medium) was prepared by mixing DMEM powder of about 1.03gm in autoclaved triple distilled water. To this 1.95gm of Hepes buffer, 3.75gm sodium bicarbonate and antibiotics like Penicillin (100µg/ml), Streptomycin (100µg/ml), Amphotericin-B(100µg/ml) were added. This was the amount of drugs should be added in 1000ml of triple distilled water. The pH was confirmed to be 7.2-7.4 using pH meter and adjustments made if needed. It was filtered under negative pressure using 0.22µm cellulose filter. 10% FBS (Foetalbovein serum) was mixed with the medium before used for culture.

c. Maintenance of adherent breast cell lines

Adherent cells MCF-7(Michigan Cancer Foundation-7) was cultured in tissue culture flasks. The cells were disaggregated by Trypsinization and sub cultured when the monolayer reached about 70% confluency. Cells were also cryopreserved at -80° C. With an inverted microscope, degree of confluency of the cell monolayer was assessed and the absence of bacterial and fungal contaminants was confirmed. Spent medium was removed. Cells were washed with PBS-EDTA (Phosphate buffered saline-Ethylene diamine tetra acetic acid) for removing all the traces of serum. Trypsin was applied on to the cell monolayer, and the flask was swirled to cover the monolayer with Trypsin. Flask was incubated at 37° C for 2-3 minutes. The Flask was examined under the inverted microscope to ensure uniform detachment of the cells. 1-2 ml of medium was added to the flask as fast as possible to reduce the Trypsin induced stress, and the contents of the flask transferred to a centrifuge tube. Cells were then centrifuged at 1500 rpm to 2000 rpm, for 10 minutes. The supernatant was discarded, and the cells were re-suspended in minimum volume of medium. Cells were counted using a Haemocytometer and used for subculture, storage and experimental purposes.

Invitro Cytotoxicity on MCF-7 cells by MTT method

The cells were harvested, counted and seeded (5000 cells/well) in 96 well plates and PBS was added to the outer wells. DMEM is added to all the wells mixed with 10% FBS. After 24 hours of incubation at 37°C in 5% CO_2 incubator to allow cell attachment. Then the media were removed. Cultures were treated with test sample *Boerhaavia diffusa L*. in different concentrations such as 50µg/ml, 100µg/ml, 200µg/ml, 400µg/ml, 800µg/ml. Again medium was added along with 10% FBS. Untreated cancer cells served as negative control. The plates were then incubated at two stages for 24 and 48hours. On completion of each stage of incubation, media were removed without disturbing the cells and to each well, 100µl of 1mg/ml solution of MTT were added. Plates were then incubated for 2hours in dark at 37ºC. 100µl of lysis buffer was added to each well and the plates were further incubated for 4hours in dark in a 37ºC incubator and absorbance was read using ELISA multi plate reader at 570nm. Triplicates were set up for each concentration. (Fig.6) The percentage of growth inhibition was calculated as follows.[12]

100- Absorbance of the Drug treated cells ×100

Absorbance of untreated control cells

RESULTS

The result of Invitro Cytotoxicity assay by MTT method for *Boerhaavia diffusa* L. at 24 and 48 hours in MCF-7 cell line were as follows

OD value for test control - 1.425

During 24 hours, the test sample *Boerhaavia diffusa* L. shows cytotoxicity of about 14.2%, 20.3%, 21.4%, 27% and 31.2% in 50,100,200,400 and 800 µg/ml concentrations receptively where as in 48 hours it shows 31.2%, 36%, 42.3%, 56.5% and 65.1% in 50,100,200,400 and 800µg/ml concentrations receptively. (fig.7)

IC_{50} values was also obtained from sigma plot software for the test sample *Boerhaavia diffusa* L. and is about 295 µg/ml

The mean percentage of inhibition of test samples *Boerhaaviadiffusa* L. was statistically evaluated. The results obtained are

Table 1: Invitro Cytotoxicity of *Boerhaavia diffusa* L. in MCF-7 cell line

Test drug	Concentration µ g/ ml				48 hour		
		Mean	SD	p value	Mean	SD	p value
BD	50	14.2	1.5	<0.001	31.2	0.6	<0.001
	100	20.3	0.7		36	0.8	
	200	21.4	1.5		42.3	1.5	
	400	27.0	0.6		56.5	2.5	
	800	31.2	0.5		65.1	1.2	

* *Boerhaavia diffusa* L.

DISCUSSION

Cancer can be defined as a disease in which a group of abnormal cells grow uncontrollably by disregarding the normal rules of cell division. Breast cancer is characterized by the uncontrolled growth of abnormal cells in the milk producing glands of the breast or in the passages that deliver milk to the nipples. Each year 1- 1.5 million new cases of breast cancer are being added all over the world. For the treatment of cancer modern science has developed many drugs but this drug burdened the patient by their cost and drug induced toxic effects. Also there is a common belief that anticancer drugs produce non-selective cell killing of normal as well as cancerous tissues. So in the present era poly herbal formulation/single herbal drugs have got more importance. Many scientific researchers have drawn attention to anticancer properties of medicinal herbs. The present study is taken up an attempt to evaluate the cytotoxic effect of decoction of herbal drug *Punarnava* (*Boerhaavia diffusa* L.) in MCF-7 breast cell line. The test sample *Boerhaavia diffusa* showed maximum cytotoxicity of about 65.1% at 48 hours in 800µg/ml concentration and there is a trend showing that when the concentration and time period increases the percentage of inhibition also increases. Statistically also its significance was calculated and it indicted that the values are highly significant from concentrations 50 µg/ ml - 800 µg/ ml.

CONCLUSION

The results of present study demonstrated that the herbal drug *Punarnava* (*Boerhaavia diffusa* L) has got cytotoxicity in MCF-7 cell line. This may be due to the phytoconstituents present in the root of the drug or due to its *Dravyaprabhavam* (specific action of drug). Thus the study drug has the potential to become a good anticancer agent. However, in vivo studies have to be carried out to substantiate the in vitro results by employing different in vivo models and clinical trials for their effective utilization as therapeutic agents.

Fig.1 *Boerhaavia diffusa* L

Fig.2 Root of *Boerhaavia diffusa* L.

Fig. 3 Dried root of *Boerhaavia diffusa* L.

Fig. 4 Concentrated decoction of *Boerhaavia diffusa* L.

Fig.5 Concentrated decoction of *Boerhaavia diffusa* diluted with 1ml triple distilled water

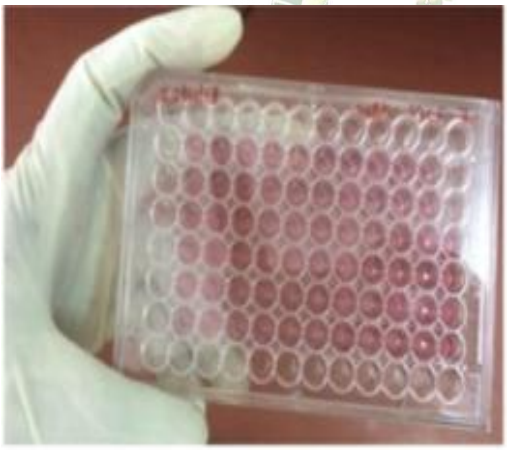

Fig.6 MTT assay plate added with test sample

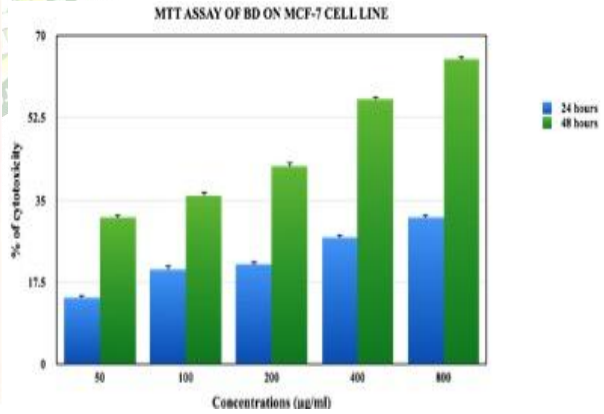

Fig. 7 Graph showing the mean percentage of inhibition of *Boerhaavia diffusa* at 24 and 48 hours

REFERENCES

1. Breast Cancer Estimated Incidence, Mortality and Prevalence Worldwide in 2012, Globocan 2012 (IARC), Section of cancer Surveillance (23.6. 2018) Available at http://globocan.iarc.fr /old/ Fact Sheets/cancers/breast-new.asp.
2. Prof. K R Srikanthamoorthy. Susrutha samhitha (Eng. Translation) Vol.1 (Nidana sthanachp.11). Varanasi; Chaukambha Orientalia; 2014. p. 532.
3. Prof. Narayana Aiyer K, Prof. Kolammal M.Pharmacognosy of Ayurvedic drugs Kerala series-1 Number 5, Trivandrum: Department of Pharmacognosy University of kerala; 1966. p. 16.
4. Baplal.G.Vaidya. Nighantu Adarsha Vol. 2, Varanasi; Chaukambha Bharathi Academy; 2005. p. 292.
5. Dr. Prakash L Hegde, Dr. Harini A, A Textbook of Dravyagunavijnana Vol. 2. Varanasi; Chaukhambha Sanskrit sansthan; 2014. p. 673.

6. Sreeja S and Sreeja S. An in vitro study on anti proliferative and antiestrogenic effects of Boerhaavia diffusa L. extracts. Journal of Ethnopharmacol 2012; 126(2): 221-225.
7. Ndubuisi Moses, Chikere Nwakanma, Bosa Ebenezeer Okoli. Cytological effects of the root extracts of Boerhaavia diffusa on root tips of Crinum jagus. Eur Asian Journal of Biosciences 2010; 4:105-111.
8. Premkumar P, Priya J, Suryavathma M. Evaluation of Antioxidant Potential of Andrographis echioides and Boerhaavia diffusa. International journal of current research 2010; 3: 59-62.
9. Meera Sumanth, Mustafa S.S..Antistress Adaptogenic and Immunopotentiating Activity of Roots of Boerhaavia diffuse in Mice. International journal of pharmacology 2007; 3(5):416-420.
10. Rupjyothi Bharathi, Mohammed R.H Azad, Jawahira Tabassum. Chemopreventive Action of Boerhaavia diffusa on PMBA- Induced skin carcinogensis in Mice. Indian Journal of physical pharmacol 2003; 47(4):459-464.
11. Saastri PP, editor. The Sarngadhara Samhitha. New Delhi: Chaukhambha publication; 2013. p. 144-5,212,306.
12. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunological Methods 1983; 65(1-2): 55-63.

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