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Screening of *Bilwadi Gutika* for Its Anticancer Action Against Gastric Carcinoma Cell Lines

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ABSTRACT

Introduction: Cancer is a group of diseases that occurs when abnormal cells grow uncontrollably or spread to other parts of the body. Despite the fact that contemporary science has developed powerful anticancer medications like cisplatin, there are many negative side effects. *Bilwadi Gutika* is an herbal drug possessing thirteen ingredients in it, among which are proven to anticancer activity. Therefore, the goal of the current study is to use the MTT assay to screen *Bilwadi Gutika*'s anticancer potential on gastric carcinoma cell lines. **Methodology:** *Bilwadi Gutika* was prepared by powdering thirteen ingredients and subjecting them to *Bhavana* (trituration) using Goat's urine. Aqueous and hydro-alcoholic extracts were prepared. MTT assay was performed to investigate the cell viability in AGS (Gastric carcinoma) cells after sample treatment. **Result:** Both aqueous and hydro alcoholic extracts showed the presence of a bioactive compounds that has anti-cancer properties. **Discussion:** Hydro alcoholic extract of *Bilwadi Gutika* at 2000 µg/ml concentration was found to have the highest cytotoxic activity on AGS (Gastric carcinoma) cell line.

INTRODUCTION

Cancer (medical term: malignant neoplasm) is a class of diseases in which a group of cells displays uncontrolled growth (division beyond the normal limits), invasion (intrusion on and destruction of adjacent tissues), and sometimes metastasis (spread to other locations in the body via lymph or blood)[1]. Factors such as lifestyle, level of physical activity, food, personal hygiene, and environmental pollution are the major contributing factors of cancer. Every person nowadays is constantly exposed to a variety of harmful compounds, the majority of which are carcinogenic in nature.[2]

Gastric cancer is the 6th most common cancer in 2020 in terms of new cases of cancer accounting 10,89,103 cases and 4th common cause of cancer death in 2020 accounting 7,68,793 deaths.[3] In India, the number of new gastric cancer cases is approximately 34,000 per year, with a male predominance (male-to-female ratio, 2:1). It was estimated that in the year 2020, approximately 50,000 new gastric cancer cases were reported annually in India. The recent nationally representative survey of cancer mortality in India indicated that gastric carcinoma was the second most common cause of cancer-related deaths amongst men and women.[4]

Kaphaja udara is one among the eight types of *Udararoga* (Enlargement of the abdomen). It produces different clinical features like heaviness of the body, lack of taste in the mouth, indigestion, body ache, numbness, oedema of the hands, feet, scrotum and thighs, nausea, sleepiness, cough, dyspnea, whitish discoloration of nails, conjunctiva, mouth, skin, urine and faeces, subjective feeling of heaviness and stillness of the abdomen, unwavering abdomen with hardness of the abdomen.[5]

By seeing the clinical features of Gastric cancer given *Ayurveda*[5] and contemporary science[4], it can be corelated to *Kaphaja Udara* and one of the *Nidana* (Cause) is found to be *Gara visha* (Concocted poison)[6]. *Gara visha* (Concocted poison) is a combination of substances either poisonous or non-poisonous. They are slowly metabolized or digested in the body, shows their effects after sometime. Now a days due to modern lifestyle each and every individual is frequently exposed to Acrylamide, Butylated Hydroxyanisole, saccharin, Organochlorine, Ethylene oxide and many more food additives and pesticides which are carcinogenic in nature.[7]

The formulation *Bilwadi Gutika* is a commonly used *Agada* formulation in clinical practice. It is mentioned in *Ashtanga Hridaya* under *Sarpa Visha pradhishedha adhyaya*. *Bilwadi gutika* along with the *chikithsa* of *sarpa visha* it is also indicated in *Gara visha* (Concocted poison).[8] It is found that all of the ingredients in *Bilwadi Gutika* are proven to possess selective anticancer activity against different cancer cells[7], so formulation may be much more effective due to synergistic action.

Cytotoxicity studies are a useful initial step in determining the potential toxicity of a test substance, including plant extracts or biologically active compounds isolated from plants.[9] The MTT assay is one of the methods used to predict the drug response in malignancies. Since the total mitochondrial activity is related to the number of viable cells, the MTT assay is broadly used to measure the in vitro cytotoxic effects of drugs on cell lines. The MTT method is simple, accurate, and yields reproducible results. Hence the present study is intended to screen anti-cancer potential of *Bilwadi Gutika* on Gastric carcinoma cell lines using MTT assay.[10]

MATERIAL AND METHODS

Preparation of extract

Aqueous extract and hydro-alcoholic extract of the formulation *Bilwadi Gutika* prepared based on API (Ayurveda Pharmacopoeia of India) standards[11]. Extraction was done using Soxhlet apparatus.

Procedure of Extraction[11, 12]

5 gms of *Bilwadi Gutika* powder was taken in a 100 ml conical flask and 50 ml of double distilled water was added (aqueous extract). Further 5 gms of *Bilwadi Gutika* powder was taken in 100 ml conical flask and 25 ml of double distilled water and 25 ml of 100 % ethanol was added for the preparation of aqueous and hydro-alcoholic extracts respectively. (Fig No. 1 & 2)

Screening of cell viability by MTT assay[13]

Materials required:

- MTT (Sigma Aldrich)

- Isopropanol
- Phosphate buffered saline (PBS)
- 96 well plate

Procedure:

A confluent cell line flask was taken and the cells were trypsinized. Cells were washed twice with Phosphate Buffered Saline (PBS) and centrifuged. The pellet was re-suspended in an appropriate medium (medium containing 10% Fetal bovine serum) and the cells were counted by using a hemocytometer. The cells were then plated in 96-well plates (10,000 cells per well) and the plate was incubated at 37^{o} C in CO_2 incubator for 24 hrs. After 24 hours, the old medium was carefully discarded from the 96-well plate. The different concentration of the test drug was prepared in suitable serum free medium and added to the different test groups and it was incubated for 48 hours at 37^{o} C in CO_2 incubator. After completion of incubation time, 20 µL of MTT dye (5 mg/mL in PBS) was added to all wells. The plate was sealed with aluminum foil and placed in a CO_2 incubator for 4 hours. Then the medium was discarded. After 4 hours, 100 µl of acidified Isopropanol was added to each well and mixed carefully by shaking. Using a multiwall plate reader, the absorbance at 540 nm (or 540 nm concerning 630 nm) was recorded.

Assessment criteria:

Effect of aqueous extract and hydro- alcoholic extract of *Bilwadi Gutika* was assessed based on percentage of viable cells calculated with the help of MTT assay.

The following formula was used to determine the percentage of viable cells:

% of viable cells = [(Test sample-blank) / (Control-blank)] x 1

RESULTS

Table No.1 - Result of extraction of *Bilwadi Gutika*

Details	Extract value
Aqueous extract of *Bilwadi Gutika*	15.08%
Hydroalcoholic extract of *Bilwadi Gutika*	22.10%

Table No. 2 - Results of MTT Assay

Conc. (µg / mL)	% Viability AQ	% Viability HY AL
Control	100	100
1	65.183	59.546
2	57.984	51.802
4	51.897	50.540
8	49.866	47.414
10	46.180	42.530
20	44.628	40.424
40	42.530	36.296
80	39.723	35.611
100	37.707	31.576
200	34.202	13.085
400	29.900	5.922
500	26.262	3.348
800	22.166	2.127
1000	14.813	1.647
2000	7.119	1.284
4000	3.934	2.062
Cisplatin 500	2.091	1.077
Cisplatin 1000	1.352	0.711

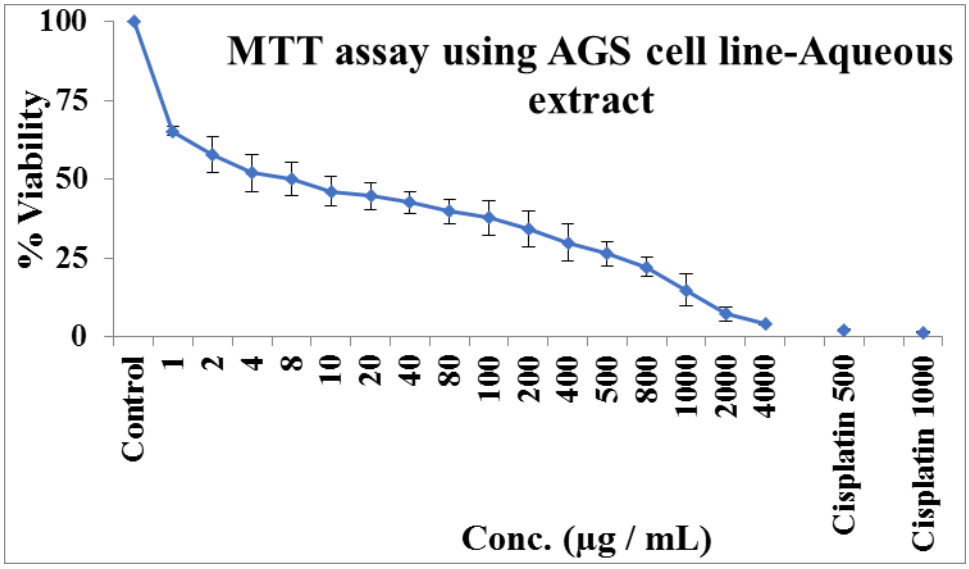

Fig No. 1: MTT assay using AGS Cell line – Aqueous extract

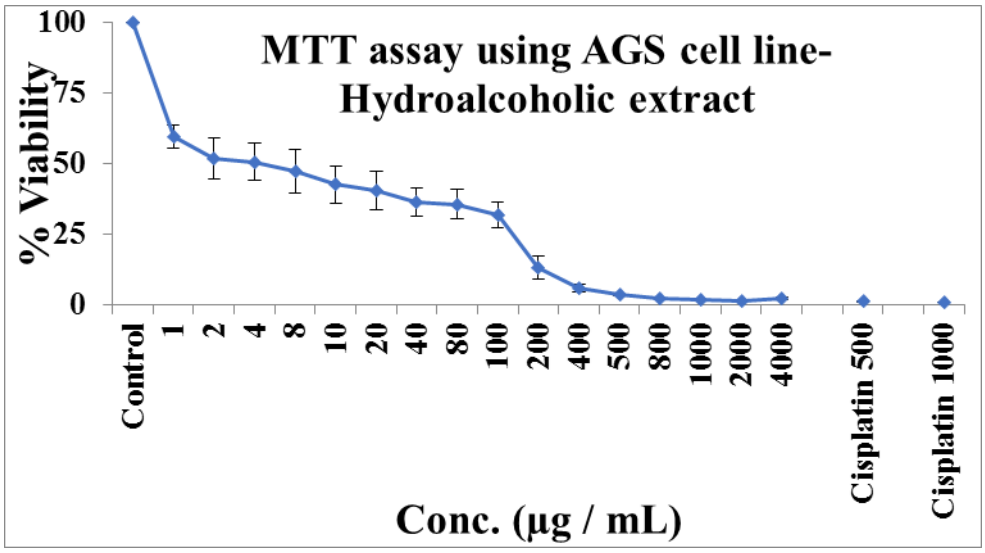

Fig No. 2: MTT assay using AGS Cell line – Hydroalcoholic extract

DISCUSSION

The cytotoxic activity of *Bilwadi Gutika* was tested on AGS cell lines using MTT assay. Aqueous and hydroalcoholic extracts were used for the cytotoxic study. The time and dose dependent responses for aqueous and hydro alcoholic extracts of *Bilwadi Gutika* against AGS cells were determined by treating cells with increasing concentrations of aqueous and hydro alcoholic extracts diluted in media. The exponentially growing AGS cells were treated with medium containing increasing concentrations of *Bilwadi Gutika* (1, 2, 4, 8, 10, 20, 40, 80,

100, 200, 400, 500, 800, 1000, 2000 and 4000 µg /ml) and dose dependent response for 4 hours determined using MTT assay.

There was a significant increasing in the percentage of cell death with increasing concentration showed significant result in both aqueous and hydro- alcoholic extract, with maximum significance in aqueous and hydro-alcoholic extract for 4000 and 2000 µg /ml doses. Hydroalcoholic extract showed better results with 98.716% cell death, as compared to aqueous extract because most of the Phytochemical dissolved in this extract which has caused cytotoxicity against cancer cells.

PROBABLE MODE OF ACTION OF *BILWADI GUTIKA*

- The predominant rasas of *Bilwadi Gutika* are *Tikta* (36%), *Katu* (32%) and *Kashaya* (29%), predominant *Gunas* are *Laghu* (44%), *Ruksha* (26%) and *Teekshna* (11%), predominant *Virya* is *Ushna* (85%) and predominant *Vipaka* is *Katu* (71%). Majority of ingredients (61%) have *Kapha vata* hara properties[14], which are opposite to the properties of *Kaphaja Udara.*

- Among 13 ingredients, *Bilwa, Surahwa, Karanja, Nata, Haritaki, Maricha, Haridra,* and *Daruharidra* are having *Vishagna* (antitoxic) action.[15]

- The *Laghu, Ruksha and Teekshna guna* makes the drug potent in action and helps for speedy action of the drug.[14]

- *Amalaki, Haritaki* and *Bilwa* [are] possessing *Rasayana* (rejuvenate) properties which are immunostimulants.[15]

- *Bilwadi Gutika* is having chemical constituents like Tannic acid, Marmelosin, Ascorbic acid, Stearic acid, Linoleic acid, Karanjin, Pongapin, Valtrate, Linarin, Atlantone, Himachalol, Taxifolin, Chebulagic acid, sorbitol, Gallic acid, Ellagic acid, Ethyl gallate, Zingiberene, Gingerol, Piperine, Piperidine, Piperlongumine, Pipernonaline, Curcumin, Camphene, Berberine, Berbamine, etc. which are proven to have different effect against cancer cells, which includes antioxidant activity, inhibition of cancer cell growth, induction of apoptosis, antitumor activity, cancer cell cytotoxicity, etc. and many were harmless to normal cells.[7]

• All ingredients are proven to have selective cytotoxic activity against cancer cells through various mechanisms like, Cell cycle arrest, increase in caspase 3 activity, Activation of tumor protein p53, Increase in ROS (reactive oxygen species), Bax-intrinsic pathway, Downregulation of Bcl 2, TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) to induce Apoptosis.[7,16] So the formulation might have caused cancer cell death by its synergistic action.

CONCLUSION

In the current study, *Bilwadi Gutika* both aqueous extract and hydro-alcoholic extracts on AGS (Gastric carcinoma) cell lines showed Cytotoxic activity based on the percentage survival of the cell in MTT assay. Comparison over the concentrations showed better results at higher concentration of 2000 µg /ml of aqueous extract and 4000 µg /ml of hydro-alcoholic extract when compared to the lower concentrations. Effect of Hydro-alcoholic extract of *Bilwadi Gutika* was found to be Significant when compared to aqueous extract of *Bilwadi Gutika* with 98.716% of cancer cell death. Standard drug Cisplatin showed more cytotoxic action compared to *Bilwadi Gutika.*

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