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AN EXPERIMENTAL EVALUATION OF *HARIDRADI LEPA* IN HUMAN CANCER CELL LINES

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Abstract: Cancer is a large group of diseases characterized by the growth of abnormal cells beyond their usual boundaries which can affect almost any part of the body. The most common cause of cancer death is due to breast, lung and liver cancer. *Haridradi Lepa* was explained in *Bhavaprakasha Nighantu* in the context of *granthi-arbuda chikitsa*. Cell culture is the best model in testing the drugs for its anticancer activity. Hence an *In vitro* anticancer activity of *Haridradi Lepa* was screened for A549 (Ca lung), HepG2 (Ca liver) and MDAMB 231 (Ca breast) cell lines. The screening was also done to evaluate the cytotoxicity effect on normal human cell lines, HEK293. It showed more cytotoxicity effect in normal cell line when compared to cancer cells.

Index Terms- *Haridradi Lepa, Arbuda*, Cancer, Cell lines.

1. INTRODUCTION

Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body.[1] Cancer is the second leading cause of death globally till date. Even though many treatment methods like chemotherapy, radiotherapy, surgical interventions, etc. are followed; it is one of the mostly challenging and dreadful disease of 21st century. It can be corelated to *Arbuda* in Ayurveda.

Ayurveda being a science of natural healing have the cure for almost all diseases. But *Arbuda* is grouped under *Asadhya vyadhi*. Nevertheless, there are many treatment procedures and medicine preparations explained by our Acharyas. *Haridradi Lepa* is one among them stated in *Bhavaprakasha Nighantu*[2] while explaining *granthi-arbuda chikitsa*. It is a herbo-mineral formulation having *Mulaka Kshara, Haridra Churna* and *Shankha Churna* as basic ingredients.

Cell lines are important tool for research into the biochemistry and cell biology of multicellular organisms. It also has uses in biotechnology. It differs from stem cells and one should not be confused with

it. The main advantage of using cell lines in research is its immortality. It can be easily cultured. They are cost-effective. It has wide range of uses from testing the effects of drugs and toxic compounds on the cells, mutagenesis, carcinogenesis to production of biological compounds. It acts as one of excellent model system for studying the normal physiology & biochemistry of cells.

The most common cause of cancer death is due to breast, lung and liver cancer.[3] Hence the study has been carried out to rule out the anticancer effect in these cancer cell lines MDAMB 231 (Ca breast), A549 (Ca lung), HepG2 (Ca liver), as well as to rule out its cytotoxic activity on normal human cell lines (Hek293).

2. AIMS & OBJECTIVES

1. To evaluate the cytotoxicity activity of aqueous extract of *Haridradi Lepa* against normal cell line using HEK293 by MTT assay.
2. To evaluate the anticancer activity of aqueous extract of *Haridradi Lepa* against A549, HepG2 and MDAMB 231 cell lines by MTT assay.

3. EXPERIMENTAL STUDY

3.1 MATERIALS

Cell Lines: HEK293 (human normal cell lines), A549 (Ca lung), HepG2 (Ca liver) and MDAMB 231 (Ca breast) cell lines were procured from NCCS Pune (CSIR lab) and grown and sub cultured at Sri Dharmasthala Manjunatheshwara Centre for Research in Ayurveda and Allied Sciences, Udupi.

3.2 METHODS

An *In vitro* cytotoxic and anticancer activities of *Haridradi Lepa* was carried out by MTT Assay.[4]

3.2.1. Preparation of Extract:

10g of dried powder of *Haridradi Lepa* was taken and 100 ml of double distilled water was added and mixed properly. The aqueous extract of *Haridradi Lepa* was prepared by running sample using Soxhlet extractor. The concentrated solvent mixture was dried using china dish and net wet of the extract was noted. The sample was stored at -20°C and further used for anticancer studies.

3.2.2. Procedure

- All the cell lines were procured from NCCS Pune and subcultured using Ham's F12k medium and fetal bovine serum.
- Around 70-80% confluent cell lines were taken and medium from the culture flasks were removed.
- The cells were separately washed twice with sterile Phosphate buffer saline (PBS) without disturbing the cells. The wash solution from the culture flasks were removed.
- Around 50-100 µl of trypsin (0.25 %) were added to the flasks and uniformly spread over the cells and the culture flasks were incubated in incubator at standard condition for approximately 2-5 minutes until the cells get starting to detach from the flasks.

- After completion of incubation time, the excess trypsin was removed, and the flasks were gently tapped and observed under inverted microscope to check the activity of trypsin on cells.
- Once the cells are detached from the flask, around 1-2 ml of fresh medium (medium with 10% fetal bovine serum) was added to the flasks separately.
- Based on the cell density around 1 to 2 ml of medium containing cells transferred to 15 ml sterile centrifuge tubes and centrifuged at around 800 to 1000 rpm for 5 to 6 minutes.
- After centrifugation, the pellets were carefully washed twice with PBS and re suspended with growth medium (medium with 10% FBS).
- About 100 µl of tryphan blue (0.04 %) was pipetted to a vial and equal volume of cell suspension was added. Both are mixed carefully and loaded to haemocytometer and counted under inverted microscope.
- After counting the cells, seed the cells to 96 well plate so that, each well having around 10,000 cells/well in 100 µl of medium.
- After completion of seeding the 96 well plate was incubated in CO_2 incubator for 24 hours.
- After 24 hours, the old medium from 96 well plate was carefully discarded.
- Cells were carefully washed once with PBS using multichannel pipette.
- Different concentrations of aqueous extract of *Haridradi Lepa* was dissolved in serum free medium and added to different test groups and incubated for 48 hours respectively. Control cells are supplemented with routine growth medium.
- Treat the cells with Cisplatin separately as a positive control.
- After completion of incubation time 20 µL of MTT dye (5mg/mL in PBS) was added to all wells in dark.
- Plate was covered with aluminium foil and incubated in CO_2 incubator at 37° C for 4 hours.
- After 4 hours, 100 µL of 0.4 N HCl and isopropanol (1:24) was added to all the wells and mixed carefully to dissolve the crystals.
- By using multi plate reader, the absorbance was recorded at 570 nm and 640 nm reference range.
- The percentage of viable cells were calculated using the formula:

% of viable cells = [(Test sample-blank) / (Control-blank)] x 100

4. OBSERVATION

Table 1: Aqueous extract of *Haridradi Lepa*

Weight of drug	10 mg
Double distilled water	100 ml
Extract value	3.773 g

Table 2: Table showing the viability of normal human cell line HEK293 at different concentrations of *Haridradi Lepa*

(µg / mL)	Mean % viability of HEK293 cell line
Control	100
1	26.10
5	19.56
10	17.12
20	16.18
40	14.71
80	13.39
100	12.90
200	11.81
500	10.91
1000	10.17
2000	9.22
5000	8.07
Cisplatin-500 µg	5.52
Cisplatin-1000 µg	2.91

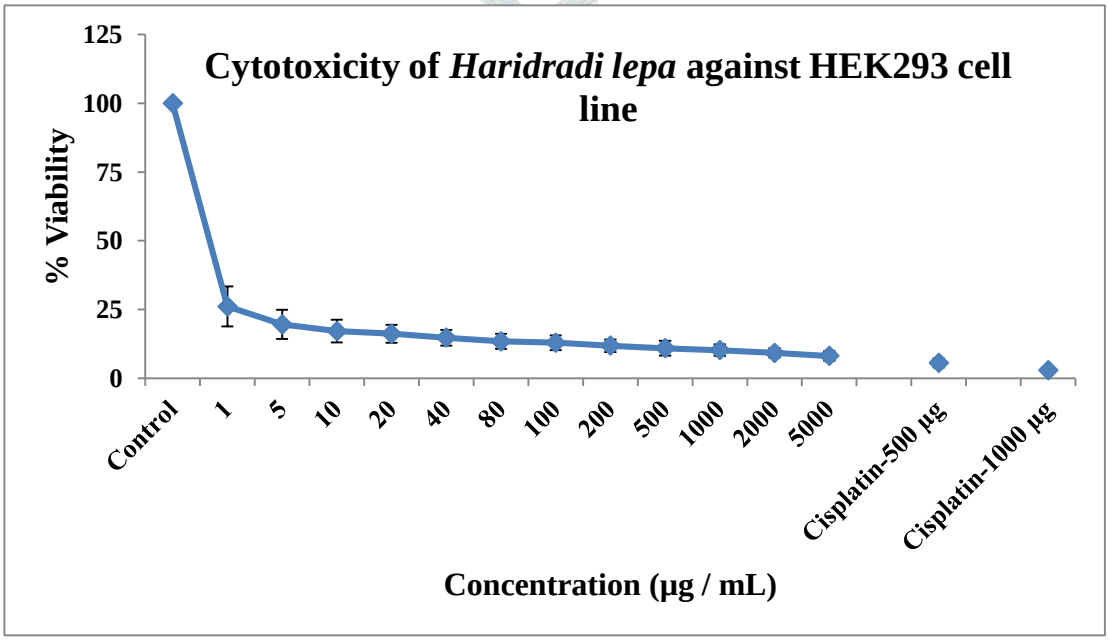

Figure 1: Cytotoxicity of *Haridradi Lepa* against HEK293 cell line

Table 3: Tabular column showing the viability of A549 cell line at different concentrations of *Haridradi Lepa*

(µg /ml)	Mean % viability of A549 cell line
Control	100
1 µg	65.73
5 µg	62.87
10 µg	62.10
40 µg	59.36
80 µg	57.77
100 µg	56.46
200 µg	53.49
500 µg	50.11
1000 µg	48.12
2000 µg	44.12
5000 µg	38.16
Cisplatin-500 µg	3.26
Cisplatin-1000 µg	0.34

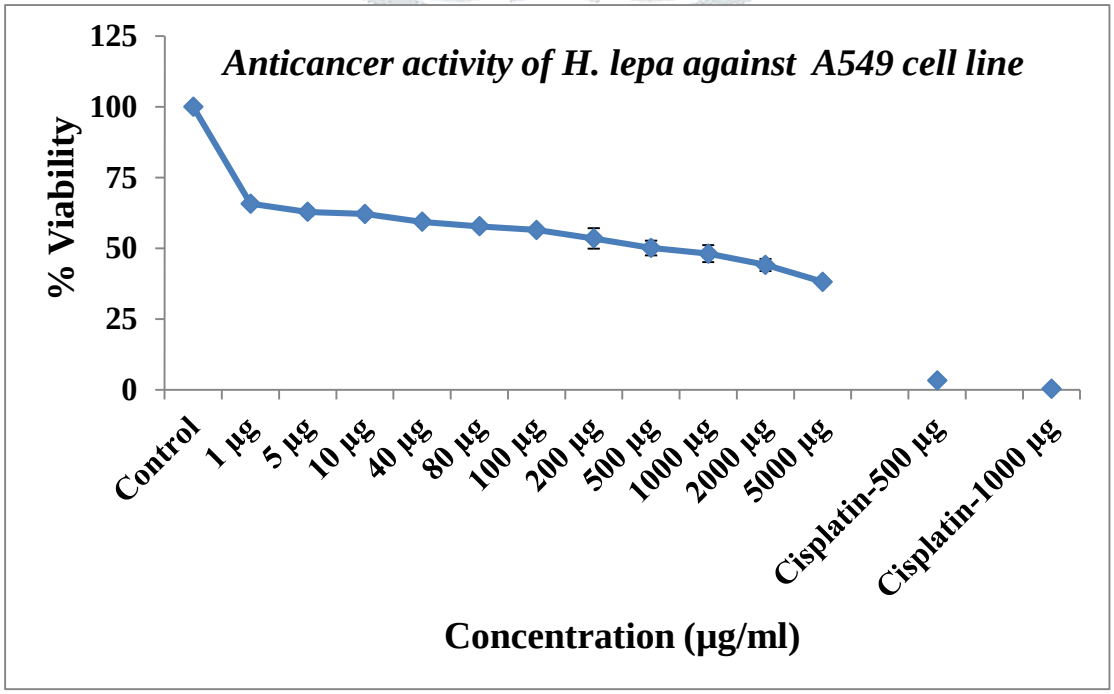

Figure 2: Anticancer activity of *Haridradi Lepa* against A549 cell line

Table 4: Table showing viability of HepG2 cell line at different concentrations of *Haridradi Lepa*

(µg /ml)	Mean % viability of HepG2 cell line
Control	100
1 µg	64.53
5 µg	63.19
10 µg	61.92
40 µg	60.89
80 µg	60.06
100 µg	58.62
200 µg	58.12
500 µg	56.37
1000 µg	55.35
2000 µg	53.79
5000 µg	51.53
Cisplatin-500 µg	3.89
Cisplatin-1000 µg	1.10

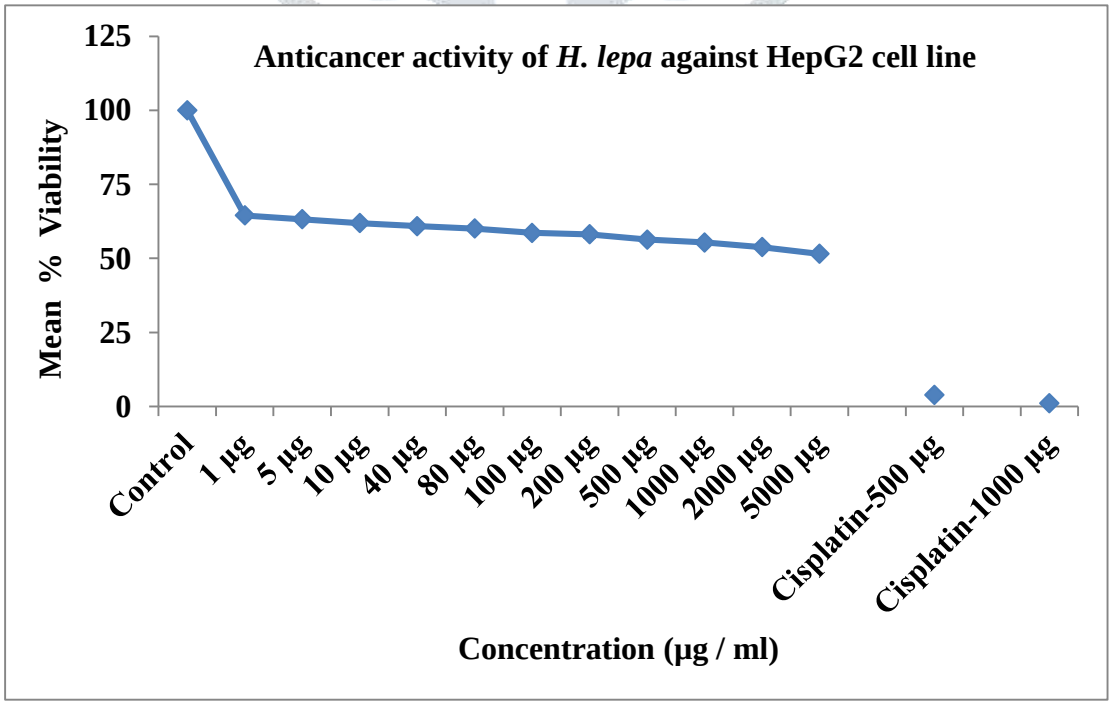

Figure 3: Anticancer activity of *Haridradi Lepa* against HepG2 cell line

Table 5: Table showing viability of MDAMB cell line at different concentrations of *Haridradi Lepa*

(µg /ml)	Mean % viability of MDAMB 231 cells
Control	100
1 µg	50.74
5 µg	47.42
10 µg	43.06
40 µg	40.45
80 µg	33.48
100 µg	30.64
200 µg	23.80
500 µg	20.06
1000 µg	14.51
2000 µg	11.12
5000 µg	6.03
Cisplatin-500 µg	3.41
Cisplatin-1000 µg	1.05

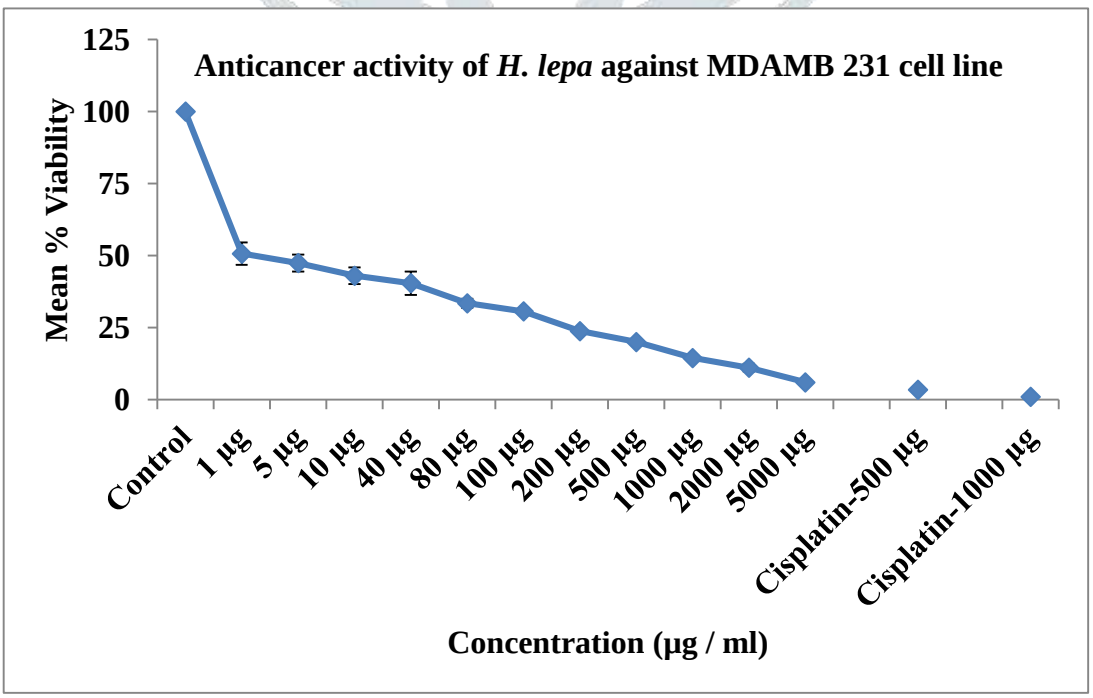

Figure 4: Anticancer activity of *Haridradi Lepa* against MDAMB 231 cell line

5. RESULTS

- In this study, different concentrations of *Haridradi Lepa* ranging from 1 µg/ml to 1000 µg/ml screened for cytotoxicity and anticancer activity for HEK293 and A549, HepG2, MDAMB 231 cell lines respectively.
- In case of HEK293 cell line, the lowest viability found was 10.91% and 10.17% after 48 hours with the maximum drug concentration of 0.5mg/ml and 1mg/ml respectively.
- In case of A549 cell line, the lowest viability value found was 50.11% and 48.12% after 48 hours with the maximum drug concentration i.e. 0.5mg/ml and 1mg/ml respectively.
- In case of HepG2 cell line, the lowest viability value found was 56.37% and 55.35% after 48 hours with the maximum drug concentration i.e. 0.5mg/ml and 1mg/ml respectively.
- In case of MDAMB 231 cell line, the lowest viability value found was 20.06% and 14.51% after 48 hours with the maximum drug concentration i.e. 0.5mg/ml and 1mg/ml respectively.

6. DISCUSSION

- *Haridradi Lepa* is having *lavana rasa, usna virya* and *laghu-ruksha-teekshna* properties. The *lekhana, chedana, bhedana*, etc. effects of *lepa* can destroy or shrink the malignant cells.
- Experimental study was carried out to check the cytotoxic and anticancer activity of *Haridradi Lepa* in different concentrations ranging from 1 to 5000 µg/ml on Hek293 (normal cell line) and MDAMB231 (Ca breast), A549 (Ca lung), HepG2 (Ca liver) human cancer cell lines.
- The cytotoxicity effect on normal cell line was more when compared to cancer cell lines.
- Among the three cancer cell lines, it was more effective in MDAMB231 cell line, then in A549 cell line and later in HepG2 cell line showing the cell viability of 14. 51%, 48.12% and 55.35% at the concentration of 1mg/ml respectively.
- Even though it showed dose dependent decrease in cell viability with respect to different doses of *Haridradi Lepa*, the dose was very high compared to the positive control Cisplatin.

7. CONCLUSION

- The study showed that the cytotoxicity effect was more on Hek293.
- The experimental study showed that there was mild to moderate anticancer activity discovered in A549, HepG2 and MDAMB231 human cancer cell lines.

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