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EFFICACY OF APAMARGA KSHARA IN CERVICAL CANCER CELL LINES

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ABSTRACT

Cancer in essence is a change in cell metabolism. Amongst the genital tract malignancies, cancer of the cervix holds the prime position followed by that of uterine endometrium, ovary, vulva, vagina and fallopian tube in that order of frequency. The *Ksharakarma* by the virtue of its *ksharana*quality has got *Chedana* and *Bhedana* effects which can destroy or desiccate the malignant cells. *Apamar-gaKshara* was prepared as per SharangadharaSamhita, Analytical study and various other parameters such as physiochemical constants were included in the present study. *An in vitro anticancer activity of ApamargaKshara was* screened on HeLa and SiHa cervical cancer cell lines. *Apamargakshara* has shown mild anticancer effect on HeLa cell line whereas it has shown moderate effect on SiHa cell line with different concentrations.

Keywords: *Apamargakshara*, *in vitro* anticancer activity, cervical cancer cell lines.

INTRODUCTION

"Cervical cancer, being a preventable disease, yet not prevented", remains a bitter reality in most of the developing world[1].

Cervical cancer continues to be the most common cancer among women in India after breast cancer. According to National Cancer registry, Approximately 1.32 lakh new cases of cervical cancer are being diagnosed and about 74,000 succumb to this disease annually accounting for one fourth of the world's cases of Cervical Cancer each year[2].

Cell culture is one of the major tools used in cellular and molecular biology, providing ex-cellent model systems for studying the normal

physiology and biochemistry of cells (e.g., metabolics), the effects of drugs and toxic compounds on the cells, and mutagenesis and carcinogenesis. It is also used in drug screening and development, and large scale manufacturing of biological compounds (e.g., vaccines, therapeutic proteins)[3].

Apamarga (AchyranthesAspera) is a pan tropical weed, found in abundance and which contains *kshara* (alkali) as a main active principle The *Kshara karma* by the virtue of its *ksharana* quality has got *Chedana* and *Bhedana* effects which can destroy or desiccate the malignant cells and at the same time by virtue of its other qualities, it is effective in eliminating the morbid *dosha* from the site of the lesion[4]. Hence, the study on efficacy of *ApamargaKshara* in Cervical cancer cell lines was undertaken.

AIM AND OBJECTIVES

- To prepare *Pratisaraniya* type of *ApamargaKshara* as mentioned in SharangadharaSamhita.
- To evaluate the efficacy of *Apamarga Kshara* in cervical cancer cell lines.

MATERIALS AND METHODS

PHARMACEUTICAL STUDY:

Preparation of *Apamarga kshara*[5]:

- *Apamargapanchanga* was taken, burnt and reduced to ash. It was allowed to cool gradually. After that the ash was carefully collected and weighed.To this four parts of water was added and stirred and was left undisturbed for overnight.
- Next day the supernatant water was collected carefully without disturbing the sediment and it was filtered using a four layered cloth. The filtrate was collected and heated until all the water content was evaporated.
- Finally white powder present at the bottom of the vessel was collected, weighed and stored in an airtight container.

ANALYTICAL STUDY:

Analytical study was carried out at Shri DharmasthalaManjunatheshwara Centre for Research in Ayurveda and Allied Sciences, Udupi.

Table a: Organoleptic characters of *ApamargaKshara*

Sample	Appearance	Colour	Taste	Touch	Odour
ApamargaKshara	Semi Solid Mass	Dull White	Saltish Bitter	Soapy	Odourless

Table b: Physico–Chemical analysis

Parameters	Result
Loss on drying	0.46%
Total ash	69.03%
Acid Insoluble Ash	0.01% w/w
Water soluble ash	68.53% w/w
pH	≈ 10

EXPERIENTAL STUDY:

Materials

- **Cell lines:** The HeLa and SiHa cell lines were procured from NCCS Pune.

Methods[6,7]

Following steps were involved in the experimental section

- The medium for HeLa and SiHa were purchased from Himedia and prepared and used as per manufacturer's instructions.
- Confluent flasks of HeLa and SiHa were trypsinized and washed with phosphate buffer saline separately.
- The cell pellet was re suspended in suitable medium with 10 % fetal bovine serum.
- Cells were counted and around 10,000 cells were seeded to 96 well plates separately and incubated at CO_2 incubator at standard condition for 24 hrs.
- After 24 hrs old medium was discarded and different concentrations of *Apamargakshara* was added to different wells in 96 well plates and incubated for 24 hrs and 48 hrs respectively.
- After completion of incubation period MTT dye was added to 96 well plates and incubated for 4 hrs in dark condition followed by addition of dimethyl sulfoxide.
- Calculation of percentage of viable cells with the following formula.

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

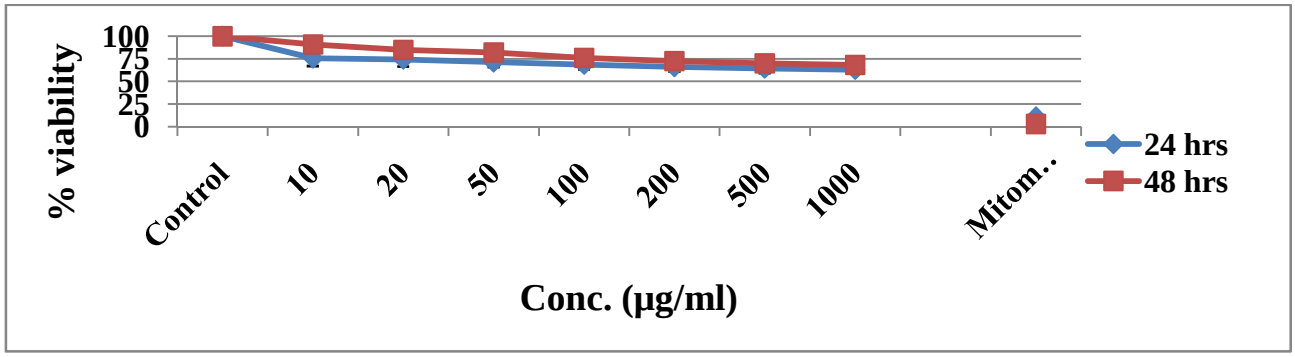
OBSERVATION

The percentage viability of HeLa cell line with different concentrations of *Apamargakshara*is shown in Table 1.

Table 1:

ApamargaKshara (µg/ml)	% cell viability	
	24 hrs	48 hrs
Control	100	100
10	75.49	91.01
20	74.34	84.96
50	71.24	82.09
100	68.52	76.00
200	65.97	72.60
500	64.29	70.12
1000	62.88	68.26
Mitomycin C (500 µg/ml)	10.80	2.95

Figure 1: The percentage viability of HeLa cell line with different concentrations of *Apamargakshara*at 24 and 48 hrs.

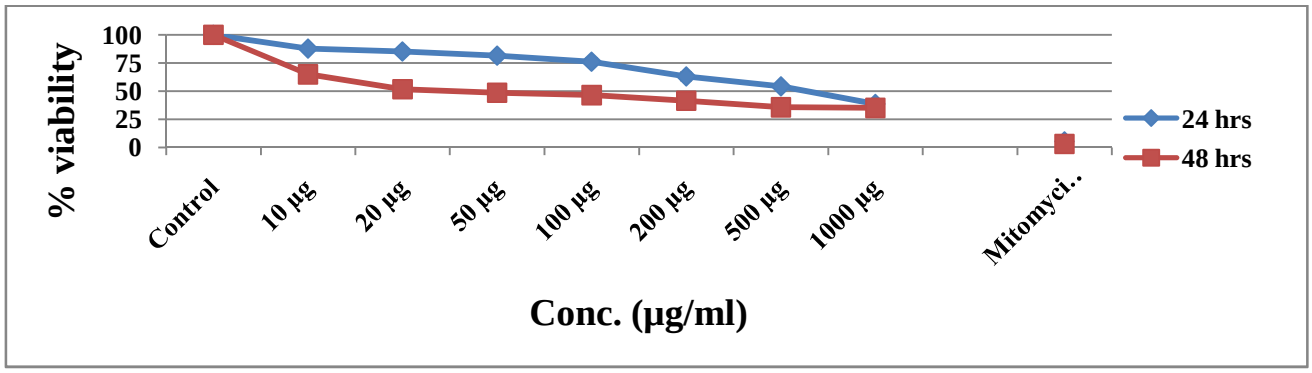


The percentage viability of SiHa cell line with different concentrations of *Apamargakshara* is shown in Table 2.

Table 2:

ApamargaKshara (µg/ml)	% cell viability	
	24 hrs	48 hrs
Control	100	100
10 µg	87.65	64.90
20 µg	85.09	51.61
50 µg	81.52	48.52
100 µg	76.11	46.37
200 µg	63.11	41.50
500 µg	54.16	35.05
1000 µg	38.56	31.85
Mitomycin C (500 µg/ml)	5.28	3.10

Figure 2: The percentage viability of SiHa cell line with different concentrations of *Apamargakshara* at 24 and 48 hrs.



RESULT

- In the present study, different concentrations of *Apamargakshara* ranging from 10 to 1000 µg/ml was selected and screened for anticancer activity with respect to 24 and 48 hrs in HeLa and SiHa cell lines.
- In HeLa cell line, Over all the maximum percentage viability value found was 75.49% after 24 hrs and 91.01 % after 48 hours with minimum drug concentration i.e., 10µg/ml.
- The lowest viability value found was 62.88% after 24 hours and 66.28% after 48 hours, with maximum drug concentration i.e., 1000 µg/ml. Over all it showed dose dependent decrease in cell viability with respect to different doses of *Apamargakshara.*
- In SiHa cell line, Over all the maximum percentage viability value found was 87.65% after 24 hrs and 64.90 % after 48 hours with minimum drug concentration i.e., 10µg/ml.
- The lowest viability value found was 38.55% after 24 hours and 35.04 % after 48 hours, with maximum drug concentration i.e., 1000 µg/ml. Over all it showed dose dependent decrease in cell viability with respect to different doses of *Apamargakshara.*
- Over all in between the two cell lines, SiHa at 48hrs incubation with respect to different concentrations of *Apamargakshara* showed maximum cell death compared HeLa cell line. In SiHa cell line 50% cell death occurred at the concentration of 20µg/ml.

DISCUSSION

- *Kshara karma* is one among the *shastiupakrama* advocated by AcharyaSushrutha for comprehensive management of *Vrana. Vranashodhana* being an important *karma* of *Kshara*, hence *Kshara* when applied in an early stage of disease manifestation may help in destruction of abnormal cells and reversing the pathology.
- The pH of the *ApamargaKshara* was 10 and which was highly alkaline. Due to its high alkalinity, it supports the definition of Alkali of being caustic and corrosive in nature and thus it may prove efficacious in destruction of pre-cancerous cell phase of Cervix.
- Experimental study was carried out to check the anticancer activity of *Apamargakshara* in different concentration ranging from 10 to1000 µg/ml on HeLa and SiHa human cancer cell lines.
- Even though it showed dose dependent decrease in cell viability with respect to different doses of *Apamargakshara*, the dose was very high compared to the positive control Mitomycin C.

CONCLUSION

Observation of experimental study has shown mild effect of *Apamargakshara* on HeLa cell lines and moderate effect of *Apamargakshara* in SiHa cervical cancer cell lines.The scope for Ayurveda in the area of oncology could be prevention, anti-cancer therapy, adjuvant to chemotherapy and improving the quality of life in advanced disease conditions.

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