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# AN EXPERIMENTAL STUDY TO EVALUATE THE EFFECT OF KRISHNA MUSALI CHOORNA AND KRISHNA MUSALI KSHARA IN CERVICAL CANCER CELL LINES

**Nayana N.*[1], Suchetha Kumari[2] and Vishwanatha[3]**

[1]PG Scholar, Department of P.G. Studies in Prasuti Tantra Evum Streeroga, Sri Dharmasthala Manjunatheshwara College of Ayurveda, Udupi.

[2]Associate Professor, Department of P.G. Studies in Prasuti Tantra Evum Streeroga, Sri Dharmasthala Manjunatheshwara College of Ayurveda, Udupi.

[3]Senior Research Officer, Sri Dharmasthala Manjunatheshwara Centre for Research in Ayurveda and Allied Sciences, Udupi.

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***Corresponding Author**
**Dr. Nayana N.**
PG Scholar, Department of P.G. Studies in Prasuti Tantra Evum Streeroga, Sri Dharmasthala Manjunatheshwara College of Ayurveda, Udupi.

## ABSTRACT

Cervical cancer known to be the most common cancer among women. Human Papilloma Virus (HPV) is considered as the main causative factor of cervical carcinoma. HPV infection is mainly transmitted through sexual contact which results in to Cervical Intraepithelial Neoplasia. Early detection and regular cervical screening will help to prevent the progression of pre-cancerous lesion in to malignancy. Based on the clinical features of CIN and cervical carcinoma we can corelate to *Arbuda*, *Granthi* or other gynaecological diseases like *Tridoshaja Yonivyapat*, *Kunapagandhi Artavadusti*, *Tridoshaja Asrugdhara* etc in Ayurveda Classics. *Krishna Musali* is one among Dasapushpa, the ten group of drugs having anticancer properties. *Krishna Musali* is mentioned in *Granthi Chikitsa*, *Kshara nirmana vidhi* etc. *Kshara* can destroy the precancerous lesion or malignant cells along with elimination of morbid *dosha* from the site of lesion by its corrosive nature. **Methodology:** HUMAN EMBRYONIC KIDNEY 293, SiHa and HeLa cell lines were obtained from NCCS, Pune. Cytotoxic effect of *Krishna Musali Choorna* and *Krishna Musali Kshara* in HEK293 and Anticancer activity in SiHa and HeLa cervical cancer cell lines were done by assessing cell viability by MTT Assay at SDM Research centre, Udupi. **Result:** *Krishna Musali Kshara* showed more cytotoxicity than

Krishna Musali *Choorna*. In SiHa cell line *Krishna Musali Choorna* and *Kshara* showed cell viability of 33.83% and 3.64 % respectively. In HeLa cell line *Krishna Musali Choorna* and *Kshara* showed cell viability of 16.21% and 6.82 % respectively. **Conclusion:** *Krishna Musali Choorna* and *Krishna Musali Kshara* showed moderate cytotoxicity against HEK293. *Krishna Musali choorna* showed more anticancer activity in HeLa compared to SiHa cell line. The *Krishna Musali Kshara* showed more anticancer activity in SiHa compared to HeLa cell line.

**KEYWORDS:** Carcinoma cervix, CIN, Cell lines, *Krishna Musali Choorna* and *Kshara*, MTT Assay.

## INTRODUCTION

Cervical cancer known to be the most common cancer among women.[1] Human Papilloma Viral (HPV) infection is the most common sexually transmitted infection in women. If HPV infection persists in infected women there is high risk of developing precancerous lesions of cervix which may even lead to Invasive Cervical Cancer (ICC). Cervical Intraepithelial Neoplasia (CIN) is the neoplasia with in the epithelial cells of the cervix. This precancerous, preinvasive phase further progress in to Cervical cancer if left untreated.[1] General description and clinical features of Arbuda only available in classics not specific or organ related.[2] Clinical features of Ca cervix include persistent vaginal discharge, bleeding, pelvic pain.[3] In advanced stages it may lead to vesicovaginal or rectovaginal fistula where the urine or faeces mixed bleeding usually noticed.[4] In initial stages of Ca cervix cauterization, cryotherapy etc. are indicated to destruct the abnormal cells. Symptoms of *Tridoshaja pradara, Tridoshaja yonivyapad, Kunapagranthi Tridoshaja arthavadushti* etc. can be correlated with the symptoms of pre-malignant and malignant condition of genital organs.[5]

*Krishna Musali* is one among the *Dasapushpa*, the group of ten sacred plants with anti-cancerous activity and having significant medicinal properties.[6] *Acharyas* included *Krishna Musali* under various *Vargas* like *Guduchyadi varga, Abhayadi varga, Musalikandadi varga* etc. *Krishna Musali* (*Curculigo orchioides*) is having *Madhuratikta rasa, ushna virya and it is vatapittahara and rasayana.*[7]

*Kshara* is a pharmaceutical preparation that kills the tissue by its *Ksharana* property. It mainly does the *Chedana* and *Bhedana karma.*[8] *Kshara* can destroy the precancerous lesion or malignant cells along with elimination of morbid *dosha* from the site of lesion by its

corrosive nature. Hence the study to evaluate the effect of *Krishna Musali Choorna* and *Krishna Musali Kshara* in Cervical cancer cell line was undertaken.

## AIMS AND OBJECTIVES

- Screening of cytotoxicity of *Krishna Musali Choorna and Kshara* against normal cell line.
- To compare the effect of *Krishna Musali Choorna* and *Kshara* in cervical cancer cell lines by *in-vitro* study.

## MATERIALS AND METHODS

### Pharmaceutical Study

*Krishna Musali Choorna* and *Krishna Musali Kshara* were prepared as per mentioned in *Sharangadhara Samhita*[9] at Rasa shastra Practical Hall, Sri Dharmasthala Manjunatheshwara College of Ayurveda, Udupi.

### Analytical Study

Analytical study *Krishna Musali Choorna* and *Krishna Musali Kshara* were carried out at Sri Dharmasthala Manjunatheshwara Centre for Research in Ayurveda and Allied Sciences, Udupi. Centre for Research and Allied Science, Udupi.

### Experimental Study

The aim of the present study is to check the cytotoxic effect of *Krishna Musali Choorna* and *Krishna Musali Kshara* in HEK 293 and anticancer effect using SiHa and HeLa cervical cancer cell lines respectively.

### Experimental source

HEK293, SiHa and HeLa cell lines were procured from NCCS Pune (CSIR lab) and sub cultured at SDM Centre for Research in Ayurveda and Allied Sciences, Udupi.

## METHODOLOGY

Screening of cytotoxicity of *Krishna Musali Choorna* and *Kshara* against normal cell line and Anticancer activity of *Krishna Musali Choorna* and *Kshara* on SiHa and HeLa cell line by MTT Assay.

**Experiments**

1. Cytotoxicity of *Krishna Musali Choorna* and *Krishna Musali Kshara* against normal cell HEK 293.
2. Anticancer activity of *Krishna Musali Choorna* and *Krishna Musali Kshara* on SiHa cell by MTT assay.
3. Anticancer activity of *Krishna Musali Choorna* and *Krishna Musali Kshara* on HeLa cell by MTT assay.

**PRINCIPLE: MTT Assay[10]**

The MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay is a simple colorimetric assay for screening cell viability, depends on cellular NAD(P)H oxidoreductase enzymes of live cells. The mitochondrial succinate dehydrogenase from live cells which reduces yellow 3-(4, 5-dimethythiazol2-yl)-2, 5- diphenyl tetrazolium bromide (MTT) to an insoluble, dark purple coloured formazan crystal. Further these formazan crystals are solubilized with suitable organic solvent and measured between 500 and 600 nm by a spectrophotometer. Since reduction of MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells (Mosmann 1983).

**Procedure**

- HEK 293 and HeLa cell line sub cultured using MEM (E) with NEAA + Na Pyruvate and fetal bovine serum and SiHa cell line sub cultured using DMEM (E) with NEAA + Na Pyruvate and fetal bovine serum.
- Around 70-80% confluent HEK cell line flask was taken and medium from the culture flask was removed.
- The cells were washed twice with sterile Phosphate buffered saline (PBS) without disturbing the cells. The wash solution from the culture flask was removed.
- Around 50-100 µl of trypsin (0.25 %) was added to flask and uniformly spread over the cells and culture flask was incubated in incubator at standard condition for approximately 2-5 minutes until cell starting detached from the flask.
- After completion of incubation time, the excess trypsin was removed and flask was 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.

- Based on the cell density around 1 to 2 ml of medium containing cells transferred to 15 ml sterile centrifuge tube and centrifuged at around 800 to 1000 rpm for 5 to 6 minutes.
- After centrifugation, the pellet was 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 hemocytometer 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.
- *Krishna Musali choorna and Krishna Musali Kshara* was dissolved in separate serum free medium and different concentration (1-4000 µg/ml) was added to different test groups and incubated for 48 hours. Control cells are supplemented with routine growth medium.
- Treat the cells with Cisplatin (500 µg/ml) 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 aluminum foil and incubated in $CO_2$ incubator at 37° C for 4 hours.
- After 4 hours, 100 µL of dimethyl sulfoxide (DMSO) 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:

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

## OBSERVATIONS AND RESULTS

**Table 1: The screening of cytotoxic activity of HEK cell line with various concentrations of *Krishna Musali Choorna* and *Krishna Musali Kshara.***

| Concentration (µg / ml) | % Cell viability | |
|---|---|---|
| | HEK293 (KMC) | HEK293 (KMK) |
| Control | 100 | 100 |
| 1 | 55.451±5.439 | 53.386±5.104 |
| 2 | 52.288±5.418 | 45.406±4.088 |
| 4 | 47.439±3.267 | 43.918±4.009 |
| 8 | 43.814±1.856 | 41.694±3.557 |
| 10 | 41.419±1.553 | 38.658±2.301 |
| 20 | 40.180±1.126 | 35.849±0.406 |
| 40 | 37.914±1.390 | 32.887±0.345 |
| 80 | 36.652±2.366 | 29.948±0.971 |
| 100 | 33.392±0.143 | 29.161±0.333 |
| 200 | 31.021±0.047 | 27.176±0.463 |
| 400 | 29.476±0.144 | 25.829±0.112 |
| 500 | 27.052±0.390 | 24.074±0.285 |
| 800 | 23.470±1.138 | 21.541±0.577 |
| 1000 | 20.433±1.389 | 18.623±0.650 |
| 2000 | 17.868±0.771 | 14.100±0.569 |
| 4000 | 11.370±2.220 | 10.160±1.500 |
| Cisplatin 500 | 2.357±0.092 | 3.187±0.010 |
| Cisplatin 1000 | 1.125±0.044 | 0.025±0.008 |

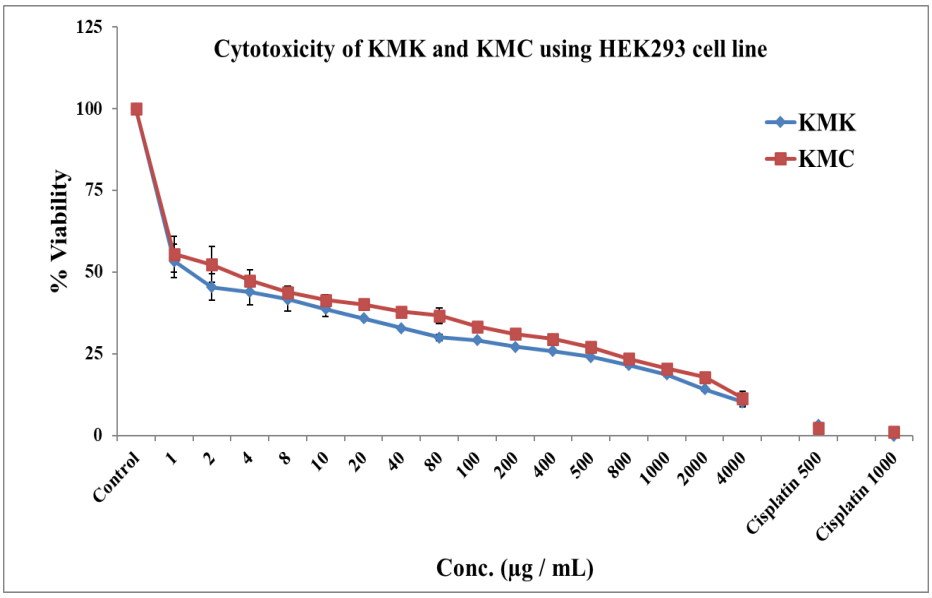


**Figure 1: The screening of cytotoxicity activity of HEK293 cell line with various concentrations of *Krishna Musali Choorna* and *Krishna Musali Kshara* at 48 hrs.**

**Results**

- Cytotoxicity of Krishna Musali Choorna and Krishna Musali Kshara showed around 54-55 % of cell viability at concentration of 1 µg/ml and it showed around 10-11 % of cell viability at higher concentration of 4000 µg/ml.

**Table 2: The anticancer screening activity of SiHa cell line with various concentrations of *Krishna Musali Choorna* and *Krishna Musali Kshara.***

| Concentration (µg / ml) | % Cell viability | |
|---|---|---|
| | SiHa (KMC) | SiHa (KMK) |
| Control | 100 | 100 |
| 1 | 89.196±7.710 | 58.672±0.114 |
| 2 | 66.639±4.369 | 51.747±0.236 |
| 4 | 59.434±0.740 | 46.206±2.411 |
| 8 | 56.815±1.394 | 45.558±2.111 |
| 10 | 53.154±1.344 | 42.142±0.054 |
| 20 | 50.364±1.759 | 38.850±1.567 |
| 40 | 47.780±1.723 | 37.312±1.957 |
| 80 | 46.623±1.325 | 36.455±1.883 |
| 100 | 46.191±1.129 | 34.896±2.131 |
| 200 | 45.305±1.306 | 31.461±1.500 |
| 400 | 43.447±0.029 | 28.755±0.719 |
| 500 | 40.776±1.097 | 27.233±0.643 |
| 800 | 40.502±1.287 | 25.877±1.026 |
| 1000 | 39.709±1.422 | 20.965±3.389 |
| 2000 | 37.095±0.172 | 16.604±4.043 |
| 4000 | 33.837±2.536 | 3.645±1.499 |
| Cisplatin 500 | 4.260±0.161 | 1.098±0.014 |
| Cisplatin 1000 | 3.892±0.147 | 0.412±0.005 |

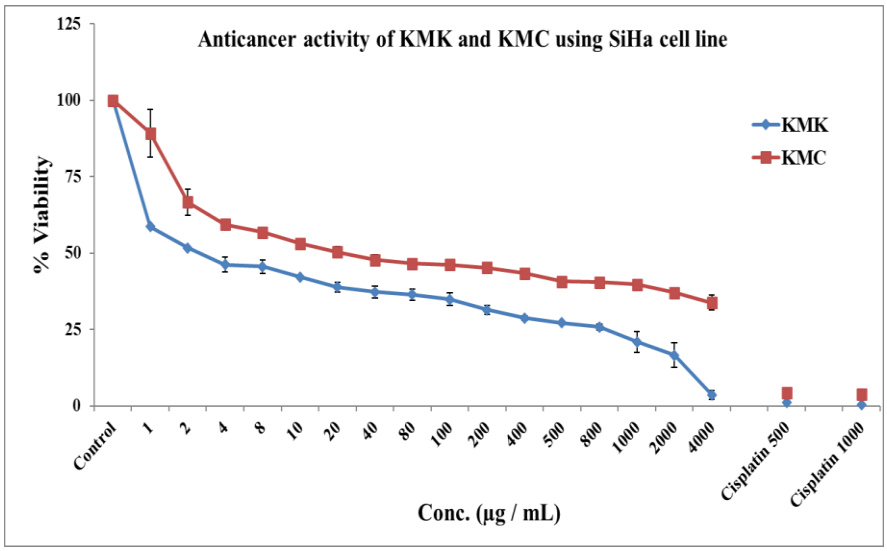


**Figure 2: The anticancer screening activity of SiHa cell line with various concentrations of *Krishna Musali Choorna* and *Krishna Musali Kshara* at 48 hrs.**

**Results**

- The anticancer activity of Krishna Musali Choorna in SiHa cell line showed around 89% at 1 µg/ml and almost 33% cell viability at concentration of 4000 µg/ml, whereas the anticancer activity of Krishna Musali Kshara at concentration of 1 µg/ml showed 58% of cell viability and almost 3% cell viability at 4000 µg/ml.

**Table 3: The anti-cancer screening activity of HeLa cell line with various concentrations of *Krishna Musali Choorna* and *Krishna Musali Kshara.***

| Concentration (µg / ml) | % Cell viability | |
|---|---|---|
| | HeLa (KMC) | HeLa (KMK) |
| Control | 100 | 100 |
| 1 | 53.228±1.959 | 50.647±0.726 |
| 2 | 51.085±1.090 | 48.134±1.280 |
| 4 | 49.477±1.804 | 43.694±0.983 |
| 8 | 46.469±0.647 | 40.862±2.671 |
| 10 | 41.630±3.358 | 39.271±1.463 |
| 20 | 39.063±5.110 | 38.018±1.928 |
| 40 | 36.723±4.209 | 33.182±1.777 |
| 80 | 34.308±5.164 | 29.374±2.076 |
| 100 | 30.627±3.248 | 25.359±0.605 |
| 200 | 29.740±3.827 | 23.437±1.948 |
| 400 | 28.091±2.842 | 21.136±0.241 |
| 500 | 27.059±3.069 | 20.142±0.447 |
| 800 | 23.959±2.014 | 15.730±2.703 |
| 1000 | 22.446±2.086 | 9.816±2.576 |
| 2000 | 17.515±4.353 | 8.169±1.617 |
| 4000 | 16.212±5.477 | 6.823±0.615 |
| Cisplatin 500 | 1.452±0.001 | 19.305±0.685 |
| Cisplatin 1000 | 0.418±0.024 | 12.087±0.429 |

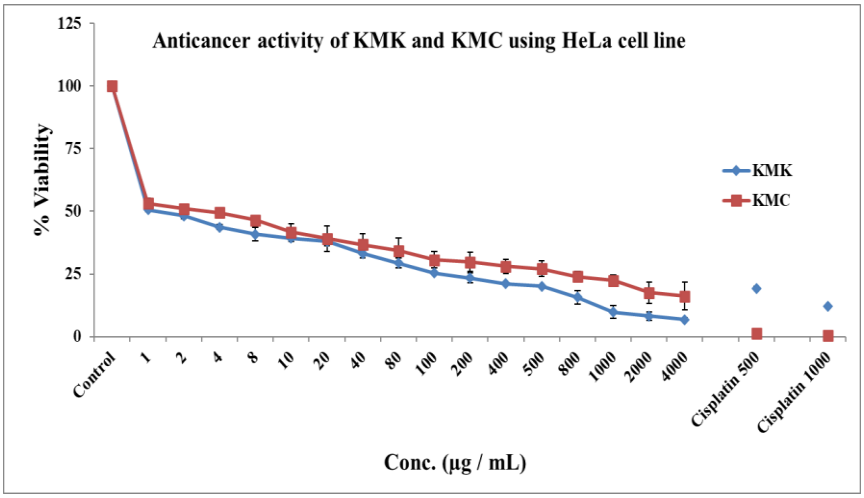


**Figure 3: The anticancer screening activity of HeLa cell line with various concentrations of *Krishna Musali Choorna* and *Krishna Musali Kshara* at 48 hrs.**

**Results**

- The anticancer activity of Krishna Musali Choorna in HeLa cell line showed around 53% at 1 µg/ml and almost 16% cell viability at concentration of 4000 µg/ml, whereas the anticancer activity of Krishna Musali Kshara at concentration of 1 µg/ml showed 50% of cell viability and almost 6% cell viability at 4000 µg/ml.

## DISCUSSION

Human Papilloma Viral infection is the most common sexually transmitted infection in women. Persistent infection by HPV has been recognized as a necessary factor for the development of CIN and Cervical cancer. HPV persistence may lead to the development of CIN and, after a latency of approximately 13 years lead to the development of invasive cervical cancer. This window period gives an opportunity for screening and early detection.

*Kshara* is a pharmaceutical preparation that kills the tissue by its *Ksharana* property. It mainly does the *Chedana* and *Bhedana karma.*[9] *Kshara* can destroy the precancerous lesion or malignant cells along with elimination of morbid *dosha* from the site of lesion by its corrosive nature. *Kshara karma* is one among the *shastiupakrama* advocated by *Acharya Sushrutha* 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 *Krishna Musali choorna* was 6 which is slightly acidic and that of *Kshara* was 12 which is 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.

This experimental study was carried out to evaluate the cytotoxicity against HEK 293 and anticancer activity against SiHa and HeLa cell lines with different concentrations of *Krishna Musali Choorna* and *Kshara* ranging from 1-4000 µg/ml, Cisplatin in used as the positive control.

### HEK 293 cell line

The lowest viability found was 11.37 % for *Krishna Musali Choorna* and 10.16% for *Kshara* with maximum concentration of 4000 µg/ml.

**SiHa Cell line**

The lowest viability found was 33.83 % for *Krishna Musali Choorna* and 3.64 % for *Kshara* with maximum concentration of 4000 µg/ml. At concentration 1000 µg/ml, *Choorna* and *Kshara* showed 39.70% and 20.96% cell viability respectively. Positive control Cisplatin showed 97.84 % cell death.

**HeLa Cell line**

The lowest viability found was 16.21 % for *Krishna Musali Choorna* and 6.82 % for *Kshara* with maximum concentration of 4000 µg/ml. At concentration 1000 µg/ml, *Choorna* and *Kshara* showed 22.44% and 9.81% cell viability respectively. Positive control Cisplatin showed 93.75 % cell death.

*Krishna Musali Choorna* and *Kshara* showed moderate cytotoxicity against HEK 293. Among these *Kshara* showed more cytotoxicity. In comparison, *Kshara* showed more anticancer activity in both SiHa and HeLa cell lines. Among this *Kshara* showed more anticancer action in SiHa cell line at maximum concentration of 4000 µg/ml.

Positive control medicine Cisplatin showed more effective in SiHa than HeLa cell line at maximum concentration of 1000 µg/ml. In HeLa cell line, action of Cisplatin less compared to *Krishna Musali Kshara*, whereas in SiHa cell line, Cisplatin showed more anticancer activity.

## CONCLUSION

Early diagnosis with regular cervical cancer screening will prevent the progression of precancerous lesions in to later stages of malignancy. Generally, the cancer cell lines are taken from fully manifested carcinoma. It is considered as incurable if left untreated. *Krishna Musali Choorna* and *Kshara* were showed moderate cytotoxicity against HEK293 and anticancer activity against SiHa and HeLa cell lines. *Krishna Musali Choorna* is showing more anticancer activity in HeLa compared to SiHa cell line. *Krishna Musali Kshara* is showing more anticancer activity in SiHa compared to HeLa cell line. Hence both can be used as an alternative because it is an easily available, safe, and effective method of prevention in the early stages of carcinoma. Ayurveda can play major role in all the aspects of malignant disorders. The scope for Ayurveda in the area of oncology could be prevention rather than cure, anticancer treatment, adjuvant to chemotherapy and improving the quality of life of advanced disease conditions.

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