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A PRECLINICAL STUDY ON EVALUATION OF ANTICANCER ACTIVITY OF SNUHI KSHARA IN HUMAN COLON CANCER CELL LINE HCT-15

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ABSTRACT Snuhi (Euphorbia neriifolia Linn.) is a conventional herb used broadly in several disease conditions as indicated in classical texts of Ayurveda. As per literature review ascertained, no literature was accessible regarding anticancer activity of Snuhi Kshara. Thus, present work was designed to evaluate the anticancer activity of Snuhi Kshara in HCT-15 (Human Colon Cancer cell line). Anticancer activity was evaluated using MTT assay by % cell viability and IC50. Anticancer activity was compared with standard drug capecitabine. A positive correlation between Concentration and % cell viability was noticed. Lowest cell viability was noted at 5000 µg concentration. Results obtained through the study indicates towards anticancer activity of Snuhi Kshara.

KEYWORDS : Ayurveda Agadtantra, Snuhi (*Euphorbia neriifolia* Linn.), Snuhi Kshara (SK), Anticancer, In Vitro, MTT assay, % cell viability, IC_{50}.

1. INTRODUCTION:

The modern era is offering advancement of technologies and lifestyle along with advanced diseases. Cancer is one of the diseases showing its widespread due to over exposure to variety of carcinogens. It is leading cause of death worldwide, accounting nearly 10 million deaths in 2020.[1] Cancer is a disease in which some of the body's cells grow uncontrollably and spreads to other parts of body.[2] It can result from abnormal proliferation of any of different kind of body cells, so there are more than hundred distinct types of cancer.[3] World's most common cancers in year 2020 were - Breast, Lung, Colon and Rectum, Prostate, Skin and Stomach cancer.[4]

Due to environmental factors, sedentary lifestyle and diet, the risk of colorectal cancer has been growing over the past few years. It is a malignant neoplasm arising from mucosal lining of colon and rectum. It comprises 98% of all malignant tumours of large intestine. Colorectal cancer is the 3rd most common primary cancer in the world.[5] New cases of Colorectal cancer in 2020 were 1.93 million and associated deaths were 9,35,000.[6] Colorectal carcinoma primarily occurs in patients of age more than 50 years. [7]

The concept of using medicinal plants in the treatment of illness has acquired renewed interest of researchers in the recent times. Thousands of herbal and traditional compounds are being screened worldwide to validate their use as anti-cancerous drugs. The science of ayurveda is supposed to add a step on to the curative aspects of cancers. Various natural products are processed and used as medicine in Ayurveda system of medicine. These medicinal products are classified in different categories of dosage forms (Panchavidha Kashaya Kalpana) in Ayurvedic Pharmaceuticals. Along with Panchavidha Kashaya Kalpana, Kshara Kalpana is an astonishing process explained by Acharyas. Kshara is prepared from dried plant parts after reducing it into ash.[8] One of the benefits of Kshara is, they are superior in all surgical and para surgical processes. [9] Hence it can be used to treat the complex diseased conditions.

Persistent prevalence and death rate is due to lack of awareness and constrained treatment services. Current therapeutic modalities are limited (Surgery, Chemotherapy and Radiation therapy) with numerous side effects and are of very high cost for common man's reach. Therefore, there is an urgent need for a safe and better-quality treatment for cancer. To overcome an increasing disease rate as well as economic and health overstraining on diseased, an integrated approach is needed to manage cancer. Snuhi Kshara is an imperative and unique formulation of Upavisha Snuhi mentioned in classics of ayurveda like Rasatarangini and Ayurvedsarasangraha. [10,11] The process of making needs minimum resources in terms of components, time span and coinage. Despite of previous known anticancer activity of extracts of Snuhi (*Euphorbia neriifolia* Linn.), [12-19] till date no document is traced on pre-clinical or clinical studies related to anticancer activity of Snuhi Kshara.

Hence present study was intended to evaluate anticancer activity of Snuhi Kshara the formulation of Upavisha Snuhi in Human colon cancer cell line HCT-15.

2. MATERIALS AND METHODS:

2.1. Pharmaceutico - Analytical Study:[20]

- Snuhi Kshara was prepared as per classical method of Kshara preparation as mentioned in Sharangadhara Samhita Madhyama khanda Adhyaya 11/ 103-106.[21]
- Analytical evaluation of test drug Snuhi Kshara (SK)[22]:
  a. Organoleptic tests: Color, Odor and Taste
  b. Physicochemical parameters: Physicochemical analysis was done as per guidelines given in Ayurvedic Pharmacopoeia of India. The parameters assessed were; pH, Loss on drying, Acid Insoluble Ash, Sodium assay, Potassium assay, Iron assay.
  c. Chromatography: The chromatographic evaluation of Snuhi Kshara (SK) was done by thin layer chromatographic (TLC) techniques.

2.2 Experimental Study:

1. HCT-15 cell line was received from NCCS (National Centre for Cell Science); cells were passaged for two times before using in the test. Cell culture was done to get the required cells for In Vitro experimental study.

2. In Vitro (Cell line) Study was performed at Crystal Biological Solutions, Pune [(CPCSEA Registration No. 2030/PO/RcBiBt/S/18/ CPCSEA) Study No: CRY/2021/043].

3. Capecitabine was used as a standard comparator. Double distilled water was used as vehicle for both test drug and comparator drug.

4. RPMI 1640 with 2mM L- glutamine was used as media with 10% filtered fetal bovine serum and 1% penicillin- streptomycin solution.

5. 100 µL of treatment medium containing either the appropriate

concentration of sample extract, or the negative control, or the positive control was added per well in triplicate

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6. Five different concentrations of the test item extract and the reference item were used in the assay.

7. Again plates were incubated for 24 hrs. and 48 hrs. (5 % CO2, 37 °C, > 90 % humidity). After 24 hrs. and 48 hrs. of incubation treated culture was carefully removed from each well.

8. Again 100 µL of fresh medium and 10 µL of MTT reagent was added and plates were incubated for 3 hrs. After incubation period MTT reagent and media was removed completely and 100 µL of dimethyl sulphoxide was added to dissolve the crystals of formazan.

9. Absorbance was measured at 570nm on ELISA plate reader.

2.3. Evaluation of % Cell Viability:

% Cell Viability was calculated using formula -

% Cell Viability = Mean absorbance of treatment / Mean Absorbance of Control ×100.

2.4. Statistical Analysis:

Relationship between mean % viability values with conc. in both test group and reference group was analysed using Pearson's Correlation Coefficient. It indicated a very strong, negative and significant (P <0.05) linear relationship between mean % viability values with conc. Paired and unpaired t- test was done for within the group and between the group comparison.

3. OBSERVATIONS AND RESULTS:

Highest viability of cells for Snuhi Kshara was observed at concentration 1000 µg (77.77%) and lowest viability was noted at 5000 µg (56.56%) at 24 hrs. For 48 hrs. % cell viability was 77.86% at 1000 µg and 57.83% at 5000 µg of concentration. Standard drug capecitabine showed 50.21% cell viability at 500 µg concentration at 24 hrs. and 50.30% viability at 48 hrs. for similar concentration. IC_{50} value of Snuhi Kshara for 24 hrs. and 48 hrs. was 2941.18 and 2906.98 respectively. IC_{50} value for reference drug remained same at both timelines i.e., 326.80.

Table No. 1: Test Drug Cell Viability at 24 and 48 hrs

Sr. No.	Concentrations	24 hrs.		48 hrs.	
		% Cell Viability	IC50	% Cell Viability	IC50
1.	5000	56.56	2941.18	57.83	2906.98
2.	4000	59.08		59.61	
3.	3000	63.62		64.86	
4.	2000	73.28		74.21	
5.	1000	77.77		77.86	

Table No. 2: Standard Drug Cell Viability at 24 and 48 hrs.

Sr. No.	Concentrations	24 hrs.		48 hrs.	
		% Cell Viability	Ic50	% Cell Viability	IC50
1.	500	50.21	326.80	50.30	326.80
2.	400	52.83		52.26	
3.	300	60.63		60.79	
4.	200	65.06		65.36	
5.	100	69.31		68.96	

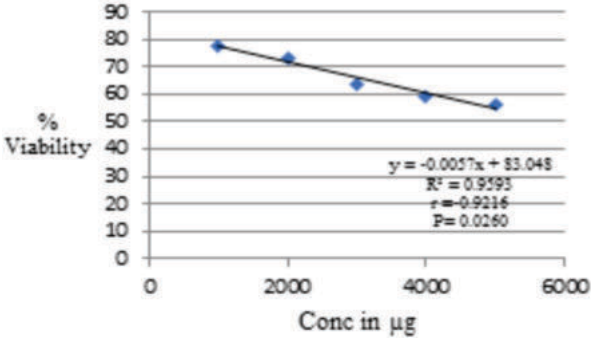

Fig. No. 1: Correlation between viability (%) and Concentration in test drug at 24 hrs.

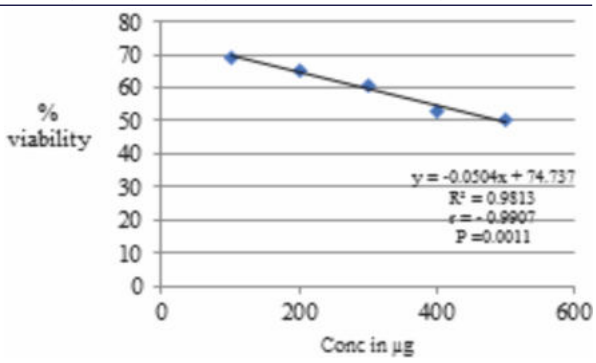

Fig. No. 2:Correlation between viability(%) and Concentration in reference drug at 24 hrs.

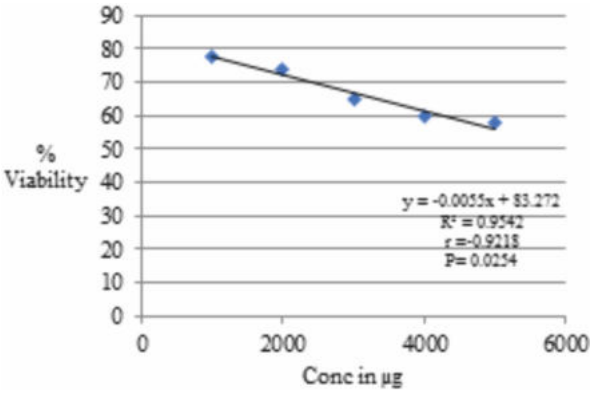

Fig. No. 3: Correlation between viability (%) and Concentration in test drug at 48 hrs.

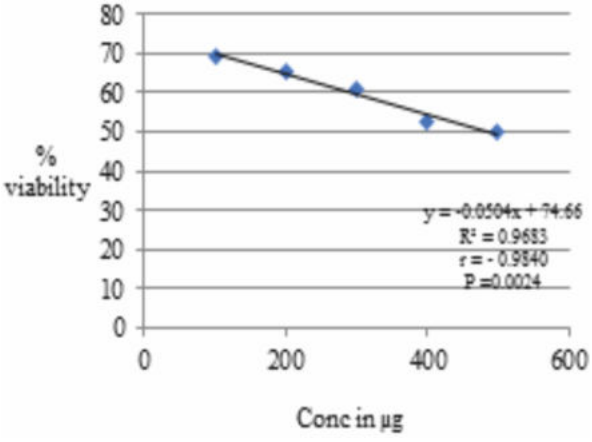

Fig. No. 4: Correlation between viability(%) and Concentration in reference drug at 48 hrs.

DISCUSSION:

Current study was designed to evaluate anticancer activity of classical preparation Snuhi Kshara (SK). Capecitabine was used as standard comparator. SK was evaluated for anticancer activity using MTT assay on HCT-15 (Human Colon cancer) cell line. In MTT assay the linear relationship between metabolically active cells and color formation is established, thus allowing an accurate quantification of changes in the rate of cell death or proliferation.[23] The biochemical mechanism behind the MTT assay involves NAD(P)H-dependent cellular oxidoreductase enzyme that converts yellow tetrazolium MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) into intracellular insoluble purple formazan [(E, Z)-5-(4,5-dimethylthiazol-2-yl)-1,3-diphenylformazan] crystals having absorbance nearly 570 nm. Hence it can be quantified by Elisa plate reader at 570 nm by dissolving formazan in DMSO. The stated mechanism takes place in metabolically active cells only. When cells die, they lose the ability to reduce MTT into formazan, thus colour formation serves as an important marker only for viable cells. [24] Two parameters accessed via performing MTT assay were % cell viability and IC_{50} that helped as major tool to evaluate anticancer activity of Snuhi Kshara.

Cell viability is a measure of the proportion of viable cells in a whole population. It is relative measure expressed as a percentage. It helps to screen drugs for their cytotoxicity and to select an anticancer drug and its dose in cancer therapy. In said work % cell viability of SK was observed at 24 and 48 hrs. timeline to rule out effect of change in time on cell viability. No major change was observed in viability with increased exposure duration. It suggests that SK shows nearly similar activity at both timelines. The decrease in cell viability with increase in concentration was noted. Decreased viability of cells is indicative of cell death caused by SK. Observations indicates that both test drug SK and Standard drug capecitabine shows anticancer activity but does not show major change in % cell viability with increased duration.

IC_{50} (Inhibitory concentration) is used early in the discovery process to evaluate the suitability and the performance of drugs. There is a monotonic relationship between the dose of the compound and the response in the assay and that there is a consistent definition of a 50% response. IC_{50} value of SK was observed at the concentration of 2941.18 µg / well at 24 hrs., minor decline in IC_{50} was observed in succeeding timeline i.e., at 48 hrs. (2906.98 µg / well). For Capecitabine IC_{50} was observed at the concentration of 326.80 µg/ well for both the timelines.

For both test drug and reference drug % of viable cells were decreased with increase in concentration at both 24 and 48 hrs. timelines. Nonsignificant change in mean % viability was observed when comparison of mean % viability between test drug and reference drug at 24 hrs. and 48 hrs. was performed. Statistical significance for comparison of IC_{50} was not established due to single observation.

Previous studies are suggestive of anticancer activity of extracts of Snuhi (*Euphorbia neriifolia Linn*.). Acharya Sushruta has narrated that Kshara removes the dushta tvakamansadi (vitiated debris of skin, flesh etc.) and also cleanse the Dosha (bodily humour). He also mentioned Kshara as useful tool in Shalya Tantra due to its actions like Chhedana (excision), Bhedana (incision), Lekhana (scrapping) etc. Integrated properties of Snuhi and Kshara may had helped to cause the cell death of HCT-15 cancer cells.

5. CONCLUSION:

Snuhi Kshara produced cell death of Human Colon Cancer cell. With increase in concentration % of viable cells were decreased. Cell death caused by Snuhi Kshara is suggestive of its anticancer activity on HCT-15 (Human Colon Cancer) cell line. Test drug showed anticancer activity at highest concentration (5000 µg) in given laboratory conditions. Current findings are helpful for creating a scope for Ayurveda in the area of oncology as an anti-cancer or adjuvant to chemotherapy and improving the quality of life in advanced disease conditions.

It also opens up an added research opportunity to recognize the exact mechanism behind cell death caused by Snuhi Kshara.

CONFLICT OF INTEREST: None.

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