

Evaluation and Effect of *Tamra Parpati Rasa* Prepared with *Asta Samskarita Parada* over Colon Cancer a Cell Line Study

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ABSTRACT

The branch of *Rasashastra* plays an integral part, in ayurveda medicine. *Rasashastra* is a science which deals with pharmacotherapeutic utilization of minerals and metals. At present, every year about 1 million new cases of colon cancer are diagnosed worldwide. It is multifactorial disease process, with encompassing genetic factor, environmental exposures (including diet) and inflammatory conditions of digestive tract. *Tamra parpati rasa* is one of the *Parpati kalpana* preparations having *Tamra bhasma* as prime ingredient. In the present study, as to fortify the preparation *Asta samskarita Parada* is taken as in classics states that the medicine prepared out of *Bhubhukshita Parada* is capable of curing chronic diseases and it is *sarvarogahara*, *rasayana*, along with *Tamra bhasma* as prime ingredient having *Param lekhana* property and *parpati* preparations are indicated in *grahani vikaras*. The study is carried out and tested for anti-cancerous activity over colon cancer with different concentrations. Drug shows maximum lyses of 38.6 % with higher dose of 1000ug/ml.

KEYWORDS: *Ashta Samskarita Parada*, *Colon Cancer*, *Cell Line Study*, *Parpati Kalpana*, *Tamra Parpati Rasa*

INTRODUCTION

Cancer is a abnormal cell growth which involving the invade and spread to other parts of body. Approximately 5-10% of cancer is inherited with genetic defects. Among them 4.4 per 100000 in men and 3.9 per 100000 in women suffer with colon cancer. Colon cancer typically affects older adults, though it can happen at any age. It usually begins with small non-cancerous (Benign) clumps of cells called polyps that form on innermost layer of intestine. Overtime some of these become malignant.

Hence cell line study plays an important role to study the biology of cancer and to test the hypothesis to improve the efficacy of treatment. In the study, Cell line study is carried out on Colo 205 cell line. Colo 205 cell line is made up of epithelial cells isolated from 1975 from ascitic fluid derived from 70 year old, Caucasian men with colon cancer. Colo 205 cell is stored at vapour phase of liquid nitrogen¹.

In the present day, the treatment available for Cancer is Surgical Management, Chemotherapy & Radiation therapy & so on, which is very costly & painful. They are also cytotoxic to normal cells which results in adverse effects on health and quality of life. Through *Ayurveda*, the management of initial stages of Carcinoma, Palliative management plays a major role in pacifying & preventing progress of disease. Hence this is an effort to analyse the efficacy of *Ayurvedic* medicine in oncology.

Rasaushadhies form an important component in *Ayurvedic* therapeutics. The innate qualities like quick action, lesser dose², acceptability, prolonged shelf life have helped *Rasaushadhies* to conquer the demand of suffering human beings. *Rasashastra* explains four unique *Murchanaa* procedures namely, *Kharaliya Rasayana*, *Parpati Rasayana*, *Kupi Pakva Rasayana* and *Pottali Rasayana*, with varying

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therapeutic activity. Among them, *Parpati Kalpas* made a revolutionary mark in the management of *Grahani*. The word *Parpati* itself indicates 'thin flake' preparations which directly influences the function of *Agni*, Thus the *Parada* and *Gandhaka* which are the base for *Parpati* are considered to be the best in treating *Grahani Vikaras* (Gastro intestinal disorders).

Tamra Parpati rasa is one of the *Parpati rasayana* having *Tamra bhasma* as a prime ingredient. To fortify the medicine *Asta samskarita Parada* is used in preparation as it is *Rasayani* and has *Sarva roga hara* property. *Tamra* is an essential element that plays a vital role in our healthy life style. *Tamra* itself is called as *Param lekha*³. *Tamra Bhasma* having *Kashaya*, *Tikta*, *Madhura*, *Amla rasa*, *Katu Vipaka*, *Ushna Virya* helps in alleviating *Pitta* and *Kaphaja dosha*, stimulates the immune system and helps to repair injured tissues and neutralize free radicals which can cause damage to cells. *Suddha Gandhaka* having *Mrutu-Jara Nashaka* and *Suddha Vatsanabha* is *Rasayana*, *Yogavahi*, *Grahi*, *Vyavayi*, *Vikasi*. Hence this drug is chosen for cell line study of colon cancer.

MATERIALS AND METHODS⁴:

Asta Samskaras of *Parada* was done as per *Rasa Hridaya Tantra*

The total quantity of *Parada* taken for *Asta Samskara*-400gms

Weight of *Parada* obtained after *Asta Samskara*-276gms

30% of loss was found after *Asta Samskaras*.

PREPARATION OF TAMRA BHASMA⁵:

Tamra bhasma was prepared as per *Rasa ratna samuchhaya*.

Initially for 1st *Putra*, Paste of *Kajjali* and *Nimbu Swarasa* was applied over *Shodhita Tamra Patra* and subjected for *Gaja puta*. For second and third *Putra*, *Chakrikas* was prepared with *Kajjali* from *Tamra churna* obtained from previous *Putra*. *Siddhi lakshanas* were obtained with 3 *gajaputas*. Hence, further *Tamra Amrutikarana* was done to remove the left over *doshas*.

Weight of *Tamra patras*-200gms

Final weight of *Tamra bhasma* obtained-180gms

PREPARATION OF TAMRA PARPATI RASA⁶:

Asta samskarita Parada and *Shodhita Gandhaka* was triturated together and prepared *kajjali*.

The prepared *Tamra bhasma* and *Shodhita Vatsanabha* was added to the *kajjali* and from this mixture *parpati* was prepared.

Table No.1:

Si. No	<i>Asta samskarita Parada</i> taken	<i>Shudda Gandhaka</i>	<i>Tamra Bhasma</i>	<i>Suddha Vatsanabha</i>	<i>Go-Ghrita</i>	Total weight obtained
1.	10 gms	20 gms	30gms	10gms	20ml	80gms

Table No.2

Si. No	Weight of Compound	<i>Parpati</i> obtained	Weight loss
1.	80gms	76 gms	4 gms

CELL LINE STUDY⁷:

MTT Assay:

Principle: The MTT Assay is a colorimetric assay for assessing cell metabolic activity. These enzymes are capable of reducing the tetrazolium dye MTT 3(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (yellow dye) to its insoluble formazan, which has a purple color. Tetrazolium dye assays can be used to measure cytotoxicity (loss of viable cells) or cytostatic activity (shift from proliferation to quiescence) of potential medicinal agents and toxic materials. MTT assays are usually done in the dark since the MTT reagent is sensitive.

MTT solution preparation:

5 mg in 1ml of Phosphate Buffer Saline (PBS – pH 7.4).

Methodology:

In vitro growth inhibition effect of test compound was assessed by colorimetric or spectro-photometric determination of conversion of MTT into "Formazan blue" by living cells.

Day 1:

1 × 10⁵ cells/ml cell suspension was seeded into each well in a 96 well micro titer plate and final volume was made upto 150 µl by adding DMEM media and incubated overnight.

Day 2:

- Dilutions of the test compounds were prepared in DMEM media.
- 100µl of the test compounds of different concentrations was added to the wells and incubated for 24 hrs, in presence of 5 % CO₂, at 37°C into CO₂ incubator.

Day 3:

- After 24 hrs, 20µl of 5 mg/ ml MTT reagent was added to the wells. The plate was kept for 4 hrs incubation in dark place at room temperature. (The plate was covered with aluminum foil, since MTT reagent is photosensitive.)
- The supernatant was carefully removed without disturbing the precipitated Formazan crystals and 10µl of DMSO was added to dissolve the crystals formed.
- The optical density (OD) was measured at wavelength of 492 nm. The study was performed in triplicates and the result represents the mean of three readings.

Formula:

Surviving cells (%) = [Mean OD of test compound / Mean OD of control] *100

Table No.3

COMPOUND	Concentration(ug/ml)	Cell viability (%)	% of Lysis
<i>Tamra Parpati rasa</i>	1000	61.4	38.6
	500	73.8	26.2
	250	81.6	18.4
	125	90.8	9.2
	62.5	92.2	7.8
Colo205 (compatibility)	1000	72.2	27.8
	500	87.2	12.8
	250	89.9	10.1
	125	91.1	8.9
	62.5	92.1	7.9

DISCUSSION:

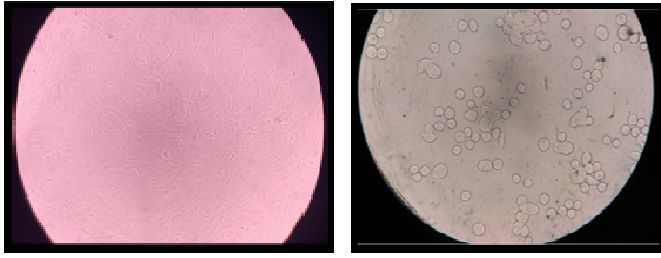
In the present study, reference for preparation of *Tamra Parpati Rasa* was taken from the *Rasa Chandhamshu*. Ayurveda classics state that *parpati*'s prepared with *Bhubhukshita Parada* become capable of curing chronic diseases. Hence, *Tamra parpati rasa* is prepared and tried in *Bhubhukshita Parada* for anti-cancerous activity. This fortified drug *Tamra Parpati Rasa* was tested on colon cancer (Colo 205) cell line study with five different concentrations with lyses viz.1000ug/ml (38.6%), 500ug/ml (26.2%), 250ug/ml (18.4%), 125ug/ml (9.2%), and 62.5ug/ml (7.8%). *Tamra Parpati Rasa* showed maximum lyses effect with higher dosage 1000ug/ml i.e. 38.6% lyses was observed. The lyses can be increase by increasing the dosage of drug.

The drug *Tamra Parpati Rasa* was tested for its compatibility on cell Colo 205 (Epithelial cell) with five different concentrations with lyses viz.1000ug/ml (27.8%), 500ug/ml (12.8%), 250ug/ml (10.1%), 125ug/ml (8.9%), and 62.5ug/ml (7.9%). Sample showed the lyses effect on epithelial cell. *Tamra* having *Param Lekhana* property it does the lyses on epithelial cell.

CONCLUSION:

Tamra Parpati rasa is *Parpati Rasayana* explained in *Rasa chandhamshu*. As Classics states that medicine prepared out of *Bhubhukshita Parada* is capable of curing chronic diseases and *Parpati kalpas* are given prime importance to *grahani vikaras*. *Tamra* also acts as *Param lekhanika*. To fortify this *Tamra parpati rasa* was tested for Anti-Cancerous activity over colon Cancer (Colo 205) with different concentration i.e- viz.1000ug/ml (38.6%), 500ug/ml (26.2%), 250ug/ml (18.4%), 125ug/ml (9.2%), and 62.5ug/ml (7.8%). *Tamra Parpati Rasa* showed maximum lyses effect with higher dosage 1000ug/ml i.e. 38.6% lyses was observed. The drug *Tamra Parpati rasa* was tested for its compatibility on cell Colo 205 (Epithelial cell) with five different concentrations with lyses viz.1000ug/ml (27.8%), 500ug/ml (12.8%), 250ug/ml (10.1%), 125ug/ml (8.9%), and 62.5ug/ml (7.9%). Sample showed the lyses effect on epithelial cell. This imply that *Asta samskarita Parada* preparation do possess higher therapeutic potency. Hence the study proved that the *Tamra parpati rasa* prepared with *Asta samskarita Parada* has promising Anti-Cancerous activity.

Microscopic examination of Compatibility
Microscopic examination of Colo 205
showing lysis



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