

Efficacy of Tamreshwara Rasa Prepared with Ashta Samskarita Parada over Liver Cancer a Cell Line Study

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ABSTRACT

RASASHASTRA is that special branch of *Ayurveda* which deals about proper usage of metals and minerals with at most care. *Rasaushadhis* are very effective in many aspects like long lasting effects, ease of palatability, quick onset of action, small dose and having better efficacy to cure the complex diseases such as cancer. Now a day's Cancer has become the biggest challenge to the scientific community over the world. Liver cancer is emerging as one of the fastest spreading cancer in India and fifth most-common cancer worldwide. *Tamreshwara Rasa* is a *Kupipakva Rasayana*, containing *Shodhita Tamra* as main ingredient along with *Shodhita Parada* and *Shodhita Gandhaka* and indicated in *Gulma*, *Shwasa* and *Sootikaroga*. In present study it is prepared with *Asta Samskarita Parada*. *Ashta Samskarita Parada* is free from *Doshas* and also makes it easily digestible, absorbable and easy for assimilation by the tissue and having *Sarvarogahara*, and *Rasayana* properties. So because of these properties, to fortify *Tamreshwara rasa*, *Ashta Samskarita Parada* was used to produce augmented anticancerous effect. So the proper scientific Validation of fortified *Tamreshwara rasa* has become one of the focused research work of new drug against Liver Cancer. Fortified *Tamreshwara rasa* was tested for Anti-cancerous activity over Liver Cancer with different concentration. Drug shows maximum lysis of 38.4% with higher dose of 1000ug/ml.

KEYWORDS: *Ashta Samskarita Parada*, *Cell Line Study*, *Kupipakva Rasayana*, *Liver cancer*, *Tamreshwara Rasa*

INTRODUCTION:

Liver cancer¹ is emerging as one of the fastest spreading cancer in India. India sees about 3-5 cases of liver cancer per 1,00,000 people which means 30,000-50,000 new cases per year. It is however, likely to be grossly under-reported as India does not have any population based data due to absence of systematic cancer registry in the country. Causative factors for liver cancer are Hepatitis B virus, Hepatitis C virus, alcoholic cirrhosis, smoking and consumption of non-veg food etc. Hep G2² is an immortalized cell line consisting of human liver carcinoma cells, derived from the liver tissue of a 15-year-old Caucasian male who had a well-differentiated hepatocellular carcinoma, which is the fifth most-common cancer worldwide. The Hep G2 cell line is commonly used in drug metabolism and hepatotoxicity studies. Hep G2 cells exhibit an

epithelial-like morphology with a modal chromosome number of 55. They are also non-tumorigenic and have high proliferation rates. Hep G2 cells are an ideal in vitro model to study the liver metabolism, drug toxicity, understanding hepatocarcinogenesis, the detection of cytoprotective and for drug targeting studies. This cell line is widely used in human cancer research of the liver and is essential for many different types of biomedical research.

Treatment modalities for liver cancer includes palliative treatment, Radiofrequency ablation, Chemotherapy and surgical management. As all these have one or other drawback, affects on body and socio-economical status of patient. Keeping a view on all these thoughts where *Ayurveda* lines of treatment found to be safer and cost-effective in palliative

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management. *Rasaoushadhis* have their own importance and major role in day-to-day practice because of their quick onset of action and higher efficacy in a smaller dosage. In classics, *Acharyas* have mentioned four varieties of *Rasa Kalpas* viz.- *Kharaliya Rasayana*, *Parpati Rasayana*, *Kupipakwa Rasayana*, and *Pottali Rasayana*. Among four *Rasa kalpas*, *Kupipakwa Rasayana* is the unique method of preparation and it deserves special importance because of its minimal dosage, maximum effect, long lasting-potency, and synergistic effects in the body. And is very potent in eliminating not only acute disease but also chronic diseases and acts as rejuvenating agents³.

*Tamreshwara Rasa*⁴ is a *Kupipakwa Rasayana*, containing *Shodhita Tamra* as main ingredient along with *Shodhita Parada* and *Shodhita Gandhaka*. *Tamreshwara Rasa* is indicated in *Gulma*, *Shwasa* and *Sootikaroga*. *Rasaushadhis* give best result in lower dosage. *Tamra* is having *Tikta*, *Kashaya Rasa*, *Ushna Veerya*, *Madhura Vipaka*. *Kaphapittashamaka*, *Puranaroganashaka*, *Yakrit-Pleeha rogahara*. As it is considered as *Param Lekhaneya dravya* used in *Kapha-Medorogas*, hence used in *Arbuda*, *Granthi* and *Gulma*. *Shuddha Parada* which is *Sarvarogahara* and *Shuddha Gandhaka* having *Mrutyu- Jaranashaka*. The medicine is

prepared out of *Astha Samskarita Parada* and other drugs by the *Kupipakwa* method which is having more potent anticancer effect was selected and compared over Liver Cancer through cell line study.

MATERIAL AND METHOD

PREPARATION OF ASHTA SAMSKARITA PARADA⁵:-

- Ashta samskara of Parada was done according to the *Rasa Hridaya Tantra*.
- The total quantity of Parada was taken for Ashta Samskara-
- Weight of Parada obtained after Ashta Samskara-
- 30% of loss was found after Ashta Samskara.

PREPARATION OF TAMRESHWARA RASA:-

The *Tamreshwara Rasa* is prepared by the method of *Bahirdhooma Kupipakwa*.

1. Ashta Samskarita Parada and *Shuddha Tamra* patra triturated and *Tamra Pishti* is prepared.
2. *Shodhita Gandhaka* added to *Tamra Pishti* and *Kajjali* is prepared.
3. This *Kajjali* is filled in *Kacha kupi*.
4. This *Kacha kupi* is placed in *Valuka Yantra* and subjected for *Kramagni* for 24 hours.
5. After *Swanga sheeta* the *Kupi* is taken out, fortified *Tamreshwara Rasa* collected from *Kantha Bhaga* and stored.

TABLE No 1

Total quantity of ingredients				Weight of Tamreshwara Rasa	Weight of Talastha Tamra Bhasma	Total weight	Wt loss	Yield
Total wt. of A.S.P	Total wt of Sh. Tamra	Total wt of Sh. Gandhaka	Total wt of Kajjali					
100gms	200gms	200gms	505gms	137gms	237gms	374gms	131gms	27%

CELL LINE STUDY⁶

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 1 ml 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:- 1×10^5 cells/ml cell suspension was seeded into each well in a 96 well micro titer plate and final volume was made upto 150 μ l by adding DMEM (Dulbecco’s Modified Eagle Medium) media and incubated overnight.

Day 2:- Dilutions of the test compounds were prepared in DMEM media. 100 μ l 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, 20 μ l 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 (Dimethyl Sulfoxide) 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.2: Table showing MTT Assay result of Tamreshwara Rasa

Cell line	Concentration of T.R (ug/ml)	Cell Viability (%)	Lysis (%)
	1000	61.6	38.4
Hep G2 cell line	500	77.4	22.6
	250	80.3	19.7
	125	86.9	13.1
	62.5	96.0	4
L929 (Compatibility)	1000	72.5	27.5
	500	73.9	26.1
	250	74.2	25.8
	125	76.2	23.8
	62.5	82.1	17.9

DISCUSSION:

Tamreshwara rasa prepared with *Ashta Samskarita Parada* has got enhanced properties like *Tikshna, Rasayana, Parama Lekhana guna*. Hence due to these properties it act as *Gulmahara, Arbudahara*. The fortified *Tamreshwara Rasa* was tested on Liver cancer (Hep G2) cell line with five different concentrations viz. 1000ug/ml, 500ug/ml, 250ug/ml, 125ug/ml and 62.5ug/ml. Sample showed maximum lysis effect with higher dose of 1000ug/ml i.e., 38.4% lysis was observed. The % of lysis can be increased by increasing dose of the drug. The drug *Tamreshwara Rasa* was tested for its compatibility on Cell line L929 (normal subcutaneous areolar adipose cells) with five different concentrations viz 1000ug/ml, 500ug/ml, 250ug/ml, 125ug/ml and 62.5ug/ml. Sample showed lysis effect on adipose cells. As *Tamra* has *Lekhaneeya Guna* hence it also does lysis of Adipose cells.

CONCLUSION:

Tamreshwara Rasa is a *Sagandha, Bahirdhooma* and *Kantastha Kupipakva Rasayana* explained in *Brihat Rasaraj Sundar*. *Tamreshwara Rasa* is indicated in *Shwasa, Sootika rogas* and *Gulma*. The fortified *Tamreshwara Rasa* was tested for Anti-cancerous activity on Liver cancer with different concentration i.e., 1000ug/ml, 500ug/ml, 250ug/ml, 125ug/ml and 62.5ug/ml. Sample shows maximum lysis of 38.4% with higher dose of 1000ug/ml. Compatibility test of *Tamreshwara Rasa* shows lysis of 27.5% with higher dose of 1000ug/ml on Adipose cells. This implies that

Ashta Samskarita Parada preparation do possess higher therapeutic potency. Hence the study proved that the *Tamreshwara rasa* prepared with *Ashta Samskarita Parada* has promising Anti- Cancerous activity.

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Microscopic structure of Tamreshwara Rasa over the Hep G2 cell

