VOL- IX | ISSUE- IV | APRIL | 2022 | PEER REVIEW e-JOURNAL | IMPACT FACTOR 7.331 | ISSN 2349-638x

To Study the Biological Activity of Shodhit Manashila on Breast Cancer Cell Lines

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1.Abstract

Cancers of the breast is among the commonest of human cancers throughout the world.Its incidence and mortality are particularly high in the developed countries.The ratio between male-female breast cancer is 1:100.The incidence of breast cancer is highest in the peri menopausal age group and is uncommon before the age of 25 years.Cancer is fast developing disease in India with high mortality rate. According to WHO total cases of cancer are upto 70 lakhs at the end of 2015 and about 5 fold growth is expected in next 10 years.

Cancer is real challenge for mankind. Whole world is fighting against cancer and always in search of any fruitful solution for it. Chemotherapy, radiation therapy, surgery all are different modalities having advantage against cancer but having disadvantages with it.

Ayurved is an ancient science. It is obvious that someone cannot find word cancer in it. But there is definitely an answer to treat cancer, to define cancer pathogenesis, to manage cancer patient with its own Chikitsatatwa. This is a science which is still alive for thousands of years. Cell line studies are safe, effective and target oriented methods for new drugs and new molecules in cancer therapies.we are selected cell line study of manshila for new molecules in cancer therapies. The requirement of drug molecule is in minimal quantity. Shodhit Manashila is biologically acting against cancer cell or not; with the help of modern method called cell-line study As per Aim of our study effect of Shodhit Manashila thus form has to be tested on cancer .to test the efficacy of Shodhit Manashila on cancer is best studied on Cancer cell lines. As per the modern pharmaceuticals when the drug for a specific it has first to studied through in-vetro (Glass study) method , then in –vivo method and then to be subjected for clinical trial. As Shodhit Manashila has been already tested for its anti-microbial activity previously this is first stage of testing Shodhit Manashila on Cancer scientifically so we have decided to go for Cancer cell line study.And result is, Shodhit Manashila (AS2S2) does not shows Specific Inhibition on Human Breast Cancer Cell.

This is usefull for clinical pratice

Keywords-Breast cancer cell,Shodhit Manashila,Arsenic compounds.

2.Introducation

Cancer is fast developing disease in India with high mortality rate. According to WHO total cases of cancer are upto 70 lakhs at the end of 2015 and about 5 fold growth is expected in next 10 years.“Rasashastra” the word itself indicating, the science mainly deals with Rasa and many other minerals, metals, herbal poisons and aquatic origin substances. Got its establishment in the medieval period when people felt its requirement owing to the changing life style which gave rise to many diseases and it flourished because of the qualities of Rasaushadis like quick action, small dose, palatability and high efficacy.

Cancer is real challenge for mankind. Whole world is fighting against cancer and always in search of any fruitful solution for it. Chemotherapy, radiation therapy,and surgery are different modalities having advantage against cancer but having disadvantages with it.

Ayurved is an ancient science. It is obvious that someone cannot find word cancer in ayurveda.But there is definitely an answer to depend the pathology and treatment modalities of cancer according to basic principal of ayurveda. This is a science which is still alive for thousands of years are itself a great task. Fundamentals, orders and basics of Ayurved are unchanged

Cell line studies are safe, effective and target oriented methods for new drugs and new molecules in cancer therapies. The requirement of drug

molecule is in minimal quantity. The cell line studies are widely used in world.

3.**Review of Literature**

Our research is a bridge between modern science and ancient science; it is way of hope for Ayurved which known to us with Ayurved fundamentals and now wants to stand with modern analytical and multi-dimensional properties so it is included with concepts of both ends.

Properties of Manashila -

Purified Manashila will have 'Katu-Tikta' Rasas and 'Snigdha-Ushna-Guru' Guna. It exhibits 'Lekhana' property. When used judiciously, it is useful in Kasa and Swasa Roga. It is found effective in all types of infectious diseases. It can be used in Agnimandya, Kshaya, Anaha, Kandu, Jwara, Visha Doshahara Rasayana. It has Tikta and Katu Rasas and Ushna Veerya and alleviates Kapha, Vata. Manashila yields more satva and eradicates Bhoota Badha, Visha, Agni mandya, Kandu, Kasa and Kshaya.

- Pharmacological and Therapeutic properties of Shodhit Manashila
  - Rasa : Tikta, Katu
  - Guna : Snigdha, Usna, Guru, Sara
  - Virya : Ushna
  - Karma : Sarva Rasayanagrya, Lekhani

O Indications of Shodhit Manashilas and its Properties :

1. Anticancer
2. Rasayana
3. Agnimandya
4. Kandu
5. Kasa
6. Kshaya

Qualities of Shodhit Manashila :

According to Rasa Tarangini, Manashila is indicated in Kasa, Swasa, Agnimandhya, Kshaya, Anaha, Kandu, Jwara, visha doshahara, rasayana.

Manahsila is considered as best among the Rasayanas. It has Tikta and Katu Rasas and Ushna Veerya and alleviates Kapha and Vata. Manahsila yields more Satva and eradicates Bhoota Badha, Visha Vikaras, Agni Mandya, Kandu, Kasa and Kshaya.

So here we can definitely give statement that it always useful in life threatening conditions and diseases with high mortality rates. Cancer is top of this list.

Most of the time a question arise how we can treat cancer patient as it is not present in samhita. This is true that word cancer is not used in samhita .But we can find answer here.

1. Dosh dhatu mala siddhant
2. Shat-kriyakala
3. Granthi varnan
4. Apachi
5. Arbuda
6. Naadivran and drushta vran

According to modern cancer is diagnosed, but in same patient we give treatment on the basis of Nidan Panchak and with references of basics of fundamentals in Samhitas. Dosh Dushya Sammurchhana when travel to Asadhyatwa, the arising Lakshanas changing. These changes are narrated by Aacharyas but in scattered manner.

One thing is definite that search of treatment or search of molecule should be based upon Ayurvediya Siddhant, terminology and methodology. Otherwise misconceptions will happen.

4.**Methodology And expirmentation**

CELL LINE STUDY :

- **Materials -**
  1. Shodhit Manshila (SM)
  2. MCF-7 human breast carcinoma cell line
  3. Adriamycin
  4. 10% fetal bovine serum
  5. 2 mM L-glutamine
  6. Dimethyl sulfoxide
  7. Sulforhodamine B solution
  8. 1% acetic acid
- **Equipments –**
  1. Microtiter plates
  2. Incubator
  3. spectrophotometer
- **Experimental Protocol :**
  - The cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine.
  - For present screening experiment, cells were inoculated into 96 well microtiter plates in 100 µL at plating densities as shown in the

study details above, depending on the doubling time of individual cell lines.

- After cell inoculation, the microtiter plates were incubated at 37° C, 5 % CO2, 95 % air and 100 % relative humidity for 24 h prior to addition of experimental drugs.
- Experimental drugs were initially solubilized in dimethyl sulfoxide at 100mg/ml and diluted to 1mg/ml using water and stored frozen prior to use.
- At the time of drug addition, an aliquote of frozen concentrate (1mg/ml) was thawed and diluted to 100 µg/ml, 200µg/ml, 400µg/ml and 800µg/ml with complete medium containing test article.
- Aliquots of 10 µl of these different drug dilutions were added to the appropriate micro titer wells already containing 90 µl of medium, resulting in the required final drug concentrations i.e.10µg/ml, 20µg/ml, 40µg/ml, 80µg/ml.

- **Endpoint Measurement-**

After compound addition, plates were incubated at standard conditions for 48 hours and assay was terminated by the addition of cold TCA.

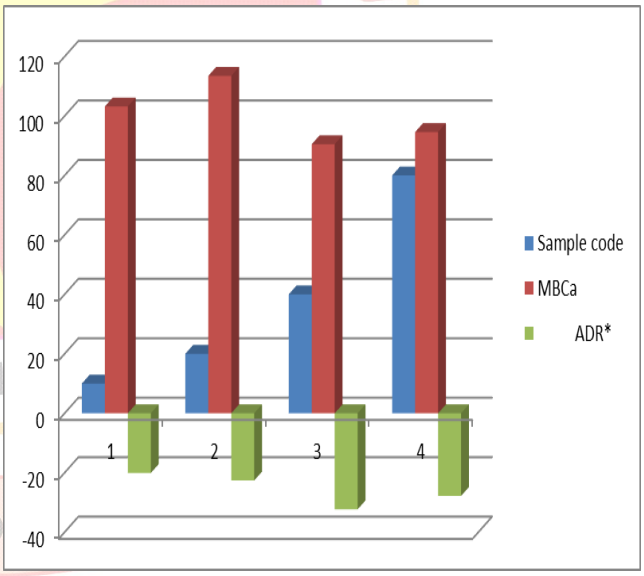
- Cells were fixed in situ by the gentle addition of 50 µl of cold 30 % (w/v) TCA (final concentration, 10 % TCA) and incubated for 60 minutes at 4°C.
- The supernatant was discarded; the plates were washed five times with tap water and air dried.
- Sulforhodamine B (SRB) solution (50 µl) at 0.4 % (w/v) in 1 % acetic acid was added to each of the wells, and plates were incubated for 20 minutes at room temperature.
- **After staining, unbound dye was recovered and the residual dye was removed by washing five times with 1% acetic acid.**
- The plates were air dried. Bound stain was subsequently eluted with 10 mMtrizma base, and the absorbance was read on an plate reader at a wavelength of 540 nm with 690 nm reference wavelength.
- Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells.
- Percent Growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells 100.
- Using the six absorbance measurements [time zero (Tz), control growth (C), and test growth in the presence of drug at the four concentration levels (Ti)], the percentage growth was calculated at each of the drug concentration levels.

prcentage growth inhibition was calculated as: [Ti/C] x 100 %.

5.Result And Discussions

EXPERIMENT - 1

Sample code	10	20	40	80
MBCa	103.2	113.5	90.5	94.6
ADR*	-20.1	-22.6	-32.3	-27.8

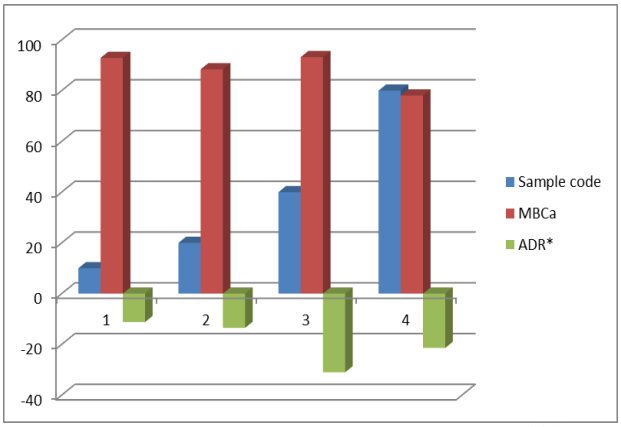


Human Breast Cancer Cell Line MCF -7

% control growth / Drug Concentration (µg)

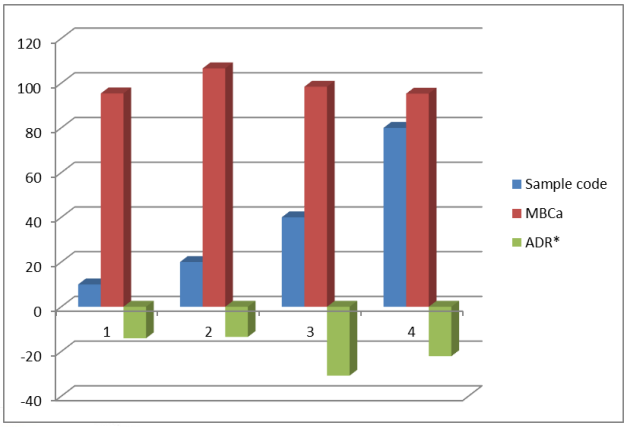
EXPERIMENT 2

Sample code	10	20	40	80
MBCa	92.9	88.4	93.2	78.1
ADR*	-11.2	-13.5	-31.0	-21.4



Human Breast Cancer Cell Line MCF -7

% control growth / Drug Concentration (µg)

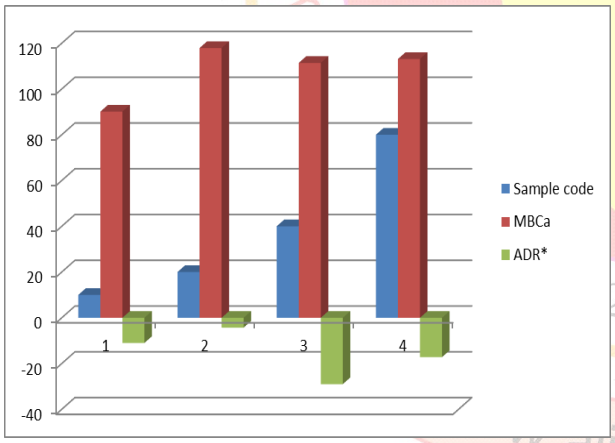


Human Breast Cell Line MFC – 7

% control growth / Drug Concentration (µg)

EXPERIMENT 3

Sample code	10	20	40	80
MBCa	90.1	117.9	111.4	113.1
ADR*	-11.0	-4.3	-29.0	-17.2



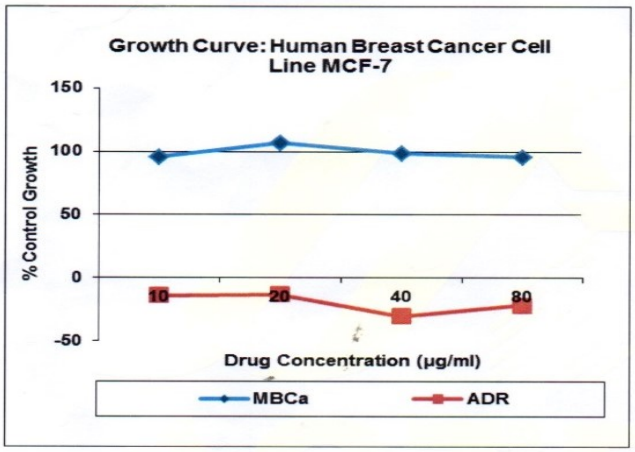
AVARAGE VALUE

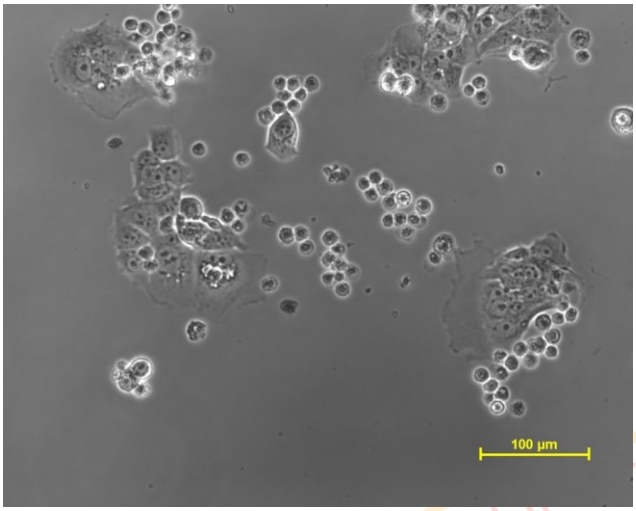
Sample code	10	20	40	80
MBCa	95.4	106.6	98.4	95.3
ADR*	-14.1	-13.5	-30.8	-22.1

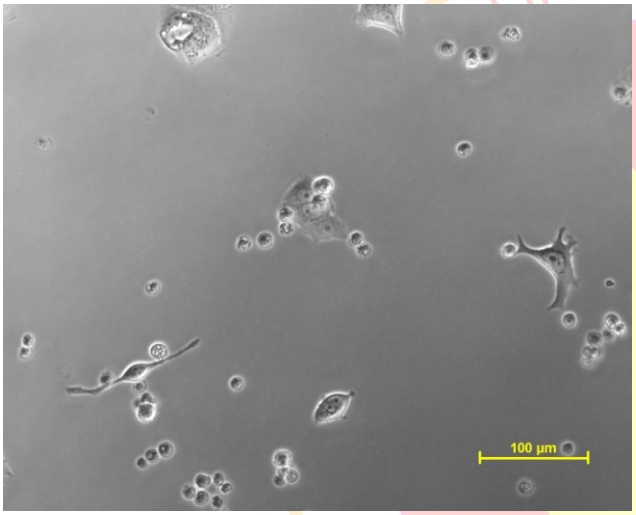
Human Breast Cancer Cell Line MCF -7

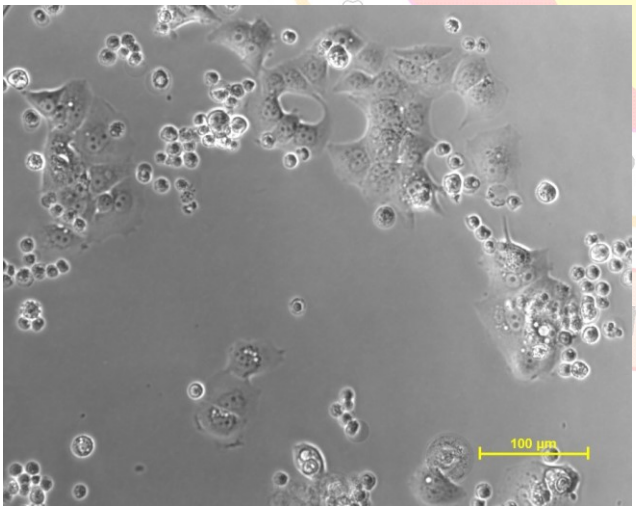
Average Values Of 3 Exper

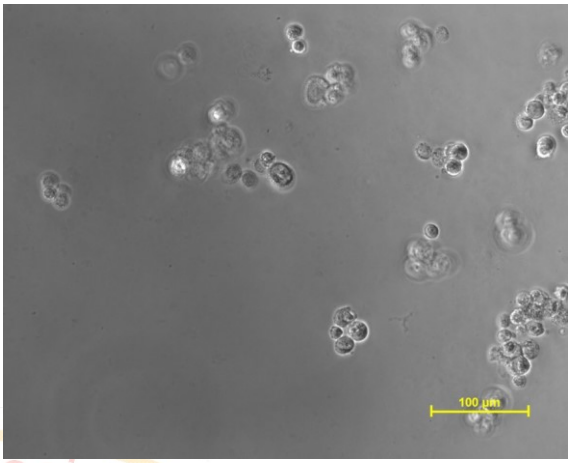
	DRUG CONCENTRATIONS (µG/ML) CALCULATED FROM GRAPH		
MCF-7	LC50	TGI	GI50*
MBCa	NE	NE	>80
ADR*	NE	<10	<10











Definitions

LC50 = Concentration of drug causing 50% cell kill

GI50 = Concentration of drug causing 50% inhibition of cell growth

TGI = Concentration of drug causing total inhibition of cell growth

ADR = Adriamycin, Positive control compound

The residual compound with ACTREC will be retained for one month from the date of this report. Enquiries regarding report will not be entertained after this date.

NE Non- evaluable data. Experiment needs to be repeated using different set of drug concentrations.

Note: Erratic data can result due to less solubility of the compound.

GI50 value of ≤ 10^-6 molar (i.e. 1 µmolar) or ≤ 10µg/ml is considered to demonstrate activity in case of pure compounds. For extracts, GI50 value ≤ 20µg/ml is considered to demonstrate activity

Yellow highlighted test values under GI50 column indicate activity.

The sample Shodhit Manashila (AS2S2) does not shows Specific Inhibition on Human Breast Cancer Cell.

6.Discussion –

1.The sample Shodhit Manashila (AS2S2) along with ADR (Adriamycine-Doxorubicin, Positive Control Drug) does not shows Specific Inhibition on Human Breast Cancer Cell : Selection of Breast cancer has been discussed previously. Thus the sample of Shodhit Manashila has been sent to ACTREC (advance Center for Treatment, Research and Education in Cancer), Kharghar, Navimumbai. For subjecting it for cell line study.

MCF -7 breast cell line has been selected for study.
Detail method of study has been discussed in Methodology.
Parameters reported – GI50 (Pure Compound),
Source of cell line – NCI, USA and NCCS, Pune.
Concentration of Drug (10, 20, 40, 80 µg/ml)
Vehicle used- Dimethyl Sulphoxiden (DMSO)
Method of testing-Sulphorhodamine B(SRB)assay.
Sample details
MBCa
MBCa + ADR (Adriamycine – Doxorubicin No Positive Control Drug)
After termination of assay results found were as follows
MCF-7 Breast Cell Line-

- The sample MBCa + ADR were inactive on Human breast cancer cell line MCF-7.

A) Breast Cancer Cell Line (MCF -7)

2.The sample Shodhit Manashila (AS2S2) does not shows Specific Inhibition on Human Breast Cancer Cell.

The study conducted to see the biological activity of Shodhit Manashila on Breast Cancer Cell lines has been carried out in Labs with the help of in-vitro cell line models of Breast cancer where diluted Shodhit Manashila is subjected directly to see the biological activity where the drug is directly get adhareted to cancer cell and shows its positive and negative action. It is might be possible that Shodhit Manashila may act positively on cancer cell actually in the body via oral route. Different biological conditions and body environment might help to digest and assimilates the drug more actively in the Body rather than acting on Cell lines. Thus the further study on Shodhit Manashila in the view of this aspect may through more light on Action of Shodhit Manashila on Breast cancer.

1) The important part we achieved from this study is that Shodhit Manashila alone does not shows specific growth on Human Breast Cancer Cell.
2) To study the biological activity of Shodhit Manashila on Breast Cancer Cell line.

7.Conclusion-

Cell Line Study –

Shodhit Manashila formed as per reference of Text subjected for following cell line study.

Breast Cancer Cell Line (MCF -7)

The sample Shodhit Manashila (AS2S2) does not shows Specific Inhibition on Human Breast Cancer Cell.

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