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Analytical And Anticancerous Activity Of *Swarna Makshika Satva Bhasma* Over Pancreatic Cancer- Invitro 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 pharmaceutico-therapeutic utilization of minerals and metals. *Swarna makshika bhasma* has been proved to rejuvenate the beta cells of pancreas in animal study. As per the classics *Swarna makshika bhasma* has been advocated in *Prameha vikaras*. Since *Satva Bhasma* is ten times more potent than *Bhasma and it is Sarvarogahara, Rasayana,* along with, *Satva* contains more Copper *(Tamra)* so this having *Param Lekhana* property, so considering the fortified therapeutic efficacy of *Swarna Makshika Satva Bhasma* over Pancreatic Cancer. Pancreatic cancer leads to many complications like Secondary Diabetes Mellitus, Jaundice etc. Pancreatic cancer is associated with poor survival and ranked as 11th most common cancer in world counting 4,58,918 new cases and causing 4,32,242 deaths-2018. Worldwide incidence and mortality of pancreatic cancer co-relate with increasing age and is slightly more common in men than women. Sample showed the maximum lysis of 31.95% in medium dose of 500ug/ml. In the present era safety and efficacy of each and every Herbo-mineral preparations becomes mandatory. These preparations should be well understood in the light of modern chemistry. These Herbo-mineral preparations are suited to physical, chemical, instrumental, macro and micro analysis before being used. This confirms the quality and safety of the drug. The sample shows the Physico- chemical parameters *Swarna Makshika Satva Bhasma* like- pH value, ash value, loss on drying, loss on ignition and solubility were within normal limit. The particle size of *Swarna Makshika Satva Bhasma* is 2,555 nm. SEM-EDX of *Swarna Makshika Satva* shows Copper (79.89%) and Iron (13.81%), SEM-EDX of *Swarna Makshika Satva Bhasma* shows Copper (38.03%) and Iron (61.97%).

Key words: Analytical Study, Celline Study, Pancreatic Cancer, *Swarna Makshika Satva Bhasma.*

I. INTRODUCTION

Analytical standards are the dimensions to evaluate a product. Due to high risk of damage to life and side effects to human body regulatory authorities paid special attention to quality of medicines and given many guidelines for safety and quality. These analytical parameters gives important information about the drug bioavailability and its effect to body. These analytical procedures are more essential in drugs and formulations. Pancreatic cancer[1] affects 10-15 per 100000 in Western populations, rising to 100 per 100000 in those over the age of 70. Men are affected twice as often as women. The disease is associated with smoking and chronic pancreatitis, Between 5 and 10% of patients have a genetic predisposition.

Approximately 90% of pancreatic neoplasms are adenocarcino- mas, which arise from the pancreatic ducts and spread early to involve local structures and regional lymph nodes. Examination reveals evidence of weight loss, Jaundice, abdominal mass due to the tumour itself, a palpable gallbladder or hepatic metastasis. A palpable gallbladder in a jaundiced patient is usually due to biliary obstruction by a pancreatic cancer (Courvoisier's sign). 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 was carried out on MIA PaCa-2 cell line[2]. In 1977, MIA PaCa-2 cells were derived from the carcinoma of a 65-year-old male. The cells have a round, epithelial morphology, and are adherent in cell culture. 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 Ca, Palliative management plays a major role in pacifying & preventing the progress of disease. Hence this is an effort to analyse the efficacy of *Swarna Makshika Satva Bhasma* in Oncology.

II. MATERIALS AND METHODS:

A) ANALYTICAL STUDY:

1] Organoleptic Analysis:

Table No. 1: showing Organoleptic Analysis of *Swarna Makshika Satva Bhasma.*

Sl.No.	Test	*Swarna Makshika Satva Bhasma*
1.	State	Fine Powder
2.	Colour	Brownish Black
3.	Taste	Tasteless
4.	Odour	Odourless

2] Physio-chemical Analysis[3-6]:

Table No. 2: showing Physio- chemical Parameters of *Swarna Makshika Satva Bhasma.*

Sl.No.	Physio- chemical Parameters	*Swarna Makshika Satva Bhasma*
1.	pH Analysis	5.3
2.	Loss on drying	0.381%
3.	Loss on ignition	14.334%
4.	Total Ash value	85.665%
5.	Acid Insoluble Ash	0.243%
6.	Water Soluble Ash	0.448%

3] Other advanced parameters:

1. **Particle Size Analysis (by Laser Diffraction Method)[7]:**

Table No. 3: showing Particle Size Analysis of *Swarna Makshika Satva Bhasma.*

Sl.No.	Parameter	*Swarna Makshika Satva Bhasma*
1	PSA	2,555 d.nm

Table No. 4: showing Particle Size distribution Report by intensity.

Results:		Size (d.nm)	Intensity (%)	Standard deviation
Z-Average (d.nm): 2555. Pdl: 1.000 Intercept: 0.858 Result Quality: Good	Peak 1	2281	100.0	318.6
	Peak 2	0.000	0.0	0.000
	Peak 3	0.000	0.0	0.000

2. **Elemental analysis by SEM-EDAX[8]:**

a) **SEM-EDAX of *Swarna Makshika Satva* were done in two ways: On Selected metals and on randomly detected metals.**

Table No. 5: showing results of SEM-EDAX of *Swarna Makshika Satva* (Selected metals)

Elements	Shell	KeV	Mass%	Sigma	Mol%	Compound	Mass%
O	-	-	20.11%	-	-	-	ND
Cu	K*	8.040	79.89%	4.25	100.00	CuO	100.00

Table No. 6: showing results of SEM-EDAX of *Swarna Makshika Satva* (Randomly detected metals).

Elements	Shell	KeV	Mass%	Sigma	Mol%	Compound	Mass%
O	-	-	18.25%	-	-	-	ND
Fe	K*	6.398	13.81%	0.98	21.67	FeO	17.77
Pb	M*	2.342	16.08%	1.19	6.80	PbO	17.32

b) SEM-EDAX of *Swarna Makshika Satva Bhasma:*

Table No. 7: showing results of SEM-EDAX of *Swarna Makshika Satva Bhasma*.

Elements	Shell	KeV	Mass%	Sigma	Atom%
Cr	K*	-	ND	-	ND
Fe	K*	6.398	61.97	2.34	64.96
Cu	K*	8.040	38.03	2.97	35.04
Zn	K*	-	ND	-	ND
Cd	L*	-	ND	-	ND
Pb	M*	-	D	-	ND

B) CELL LINE STUDY[9]:

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.

MAINTENANCE OF CELL LINES:

Cell lines of MIA PaCa-2 were procured from National center for cell Sciences (NCCS) Pune. The data sheet with sixteen short tandem repeat (STRI loci proved to be 100% matching with ATCC STR profile. After procuring the cell lines, maintenance and sub culturing of the cells was done by preparing 100nd of complete media comprising of DMEM 89ml (Himedia, Ref AL007S) FBS 10ml (Himedia RM 10432, LOT 573421), and antibiotics Ind (Himedia A002, LOT 5392281) The cells were maintained in 5% CO2 incubator and observed under inverted light microscope. All the procedure of cell culture was performed in Class II cabinet by considering all the aseptic conditions on cells reaching the 85% confluence, trypsinization was performed using trypsin (TCL007, LOT 5366/91) and sub culturing was done as per the standard protocol

III. METHODOLOGY:

During the cells in log phase of growth MTT assay was performed. The assay was designed by considering negative controls without adding compound and Cisplatin was considered as positive control. The MTT assay was performed using treated flat bottom 96 well plate. The markings was done by considering negative control, compound of *Swarna Makshika Satva Bhasma* concentrations of 500 µg/ml -15.6µg/ml. was considered. The assay was set by in triplicate wells for all concentrations of the compound to avoid any bias. Trypan blue assay was performed and Number of viable cells were counted and calculated and seeded 5 x10' cells per well in a 96 well plate. Later complete media was added to make the volume of 150 micro liters. After 24 hours of incubation in 5% carbon dioxide incubator once the cells got attached and reached log phase of growth test compound was added. The compounds of serial dilution was added to wells and incubated for 24 hours. The supernatant was discarded and washed with PBS and 200 micro liters of fresh media was added to the wells and incubated for 24 hours. Later media with 20 µl of MTT dye was added and the plate was wrapped in silver foil as MTT dye is photosensitive and incubated for four hours. The supernatant was slowly removed and discarded without disturbing the formazan crystals, 100 µl of 1% DMSO was added to dissolve the crystals and 25pl glycine buffer was added to optimize the pH by using Spectrophotometer at around 570nm reading was obtained and calculated the proliferative index by dividing the OD of test with OD of control multiplied by 100.

Formula:

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

Table No. 8: Table showing results of MTT Assay test of SMSB.

COMPOUND	Concentration (ug/ml)	Cell viability (%)	% of Lysis
Swarna Makshika Satva Bhasma	500	68.05	31.95
	250	70.1	29.9
	125	73.40	26.6
	62.5	76.59	23.41
	31.2	78.10	21.9
	15.6	94.14	5.86

Inference: By MTT assay we found that the *Swarna Makshika Satva Bhasma* compound showed cytotoxicity only at higher concentration i,e 500µg/ml. and 250 µg/ml treated on pancreatic cancer cell lines i,e MIA PaCa-2 cell lines. The IC (Inhibitor Concentration) 50 value calculated for this compound being **650.65 µg/ml.**

IV. DISCUSSION:

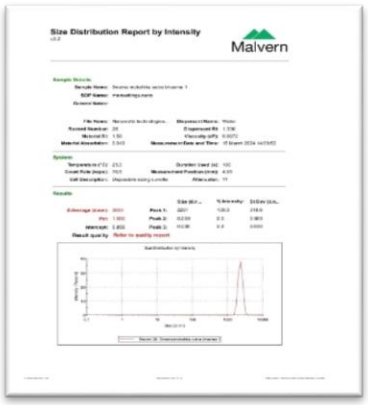
In the present study, *Swarna Makshika Satva Bhasma* was prepared as per *Rasa Tarangini*[10]. This sample shows the Physico- chemical parameters *Swarna Makshika Satva Bhasma* like- pH value, ash value, loss on drying, loss on ignition and solubility were within normal limit. The particle size of *Swarna Makshika Satva Bhasma* is 2,555 nm, as the particle size is very less so the dissolution & absorption is very fast. That indicate the quality of the *Swarna Makshika Satva Bhasma* was very good. Previous studies done over *Swarna Makshika Bhasma* have shown the concentration of Cu & Fe in the percent of 16% and 32% respectively. Considering the previous reports the Cu and Fe concentration obtained in *Satva* & *Satva Bhasma* is much logical and also inferred by reports. This fortified drug *Swarna Makshika Satva Bhasma* was tested on Pancreatic Cancer (MIA PaCa-2) cell line study with six different concentrations with lysis viz. 500ug/ml (31.95%), 250ug/ml (29.9%), 125ug/ml (26.6%), and 62.5ug/ml (23.41%), 31.2ug/ml (21.9%), 15.6ug/ml (5.86%). *Swarna Makshika Satva Bhasma* showed maximum lysis effect with lower dosage 500ug/ml i.e. 31.5% lysis was observed. The IC (Inhibitor Concentration) 50 value calculated for this compound being 650.65 µg/ml. The lysis can be increased by increasing the dosage of drug. Sample showed the lysis effect on epithelial cell. *Swarna Makshika Satva Bhasma* having *Parama Lekhana* property it does the lysis on epithelial cell.

V. CONCLUSION:

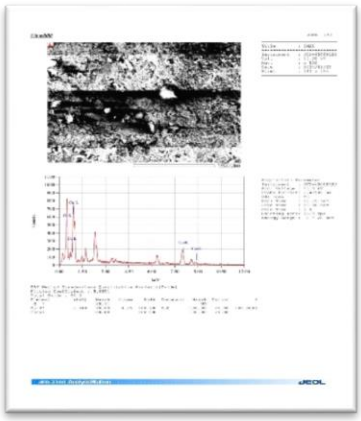
Swarna Makshika Satva Bhasma is ten times more potent than *Swarna Makshika Bhasma* so considering the fortified therapeutic efficacy of *Swarna Makshika Satva Bhasma* over Pancreatic Cancer study. Previous studies done over *Swarna Makshika Bhasma* have shown the concentration of Cu & Fe in the percent of 16% and 32% respectively. Considering the previous reports the Cu and Fe concentration obtained in *Satva* & *Satva Bhasma* is much logical and also inferred by reports. The particle size of *Swarna Makshika Satva Bhasma* is 2,555 nm, as the particle size is very less so the dissolution & absorption is very fast. That indicate the quality of the *Swarna Makshika Satva Bhasma* was very good. By MTT assay we found that the *Swarna Makshika Satva Bhasma* compound showed cytotoxicity only at higher concentration i,e 500µg/ml. and 250 µg/ml treated on pancreatic cancer cell lines i,e MIA PaCa-2 cell lines. Sample showed the maximum lysis of 31.95% in medium dose of 500ug/ml. The IC (Inhibitor Concentration) 50 value calculated for this compound being 650.65 µg/ml. The lysis can be increased by increasing the dosage of drug. Sample showed the lysis effect on epithelial cell. *Swarna Makshika Satva Bhasma* having *Parama Lekhana* property it does the lysis on epithelial cell. This imply that *Swarna Makshika Satva Bhasma* do possess higher therapeutic

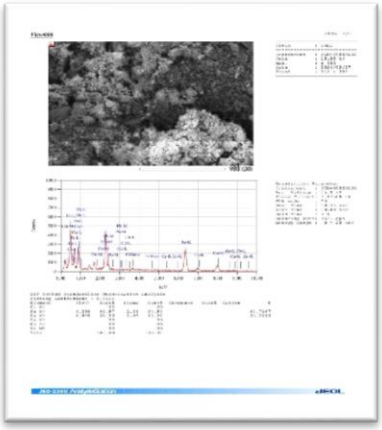
potency. Hence the study proved that the *Swarna Makshika Satva Bhasma* has promising Anti-Cancerous activity.

VI. OBSERVATIONS:



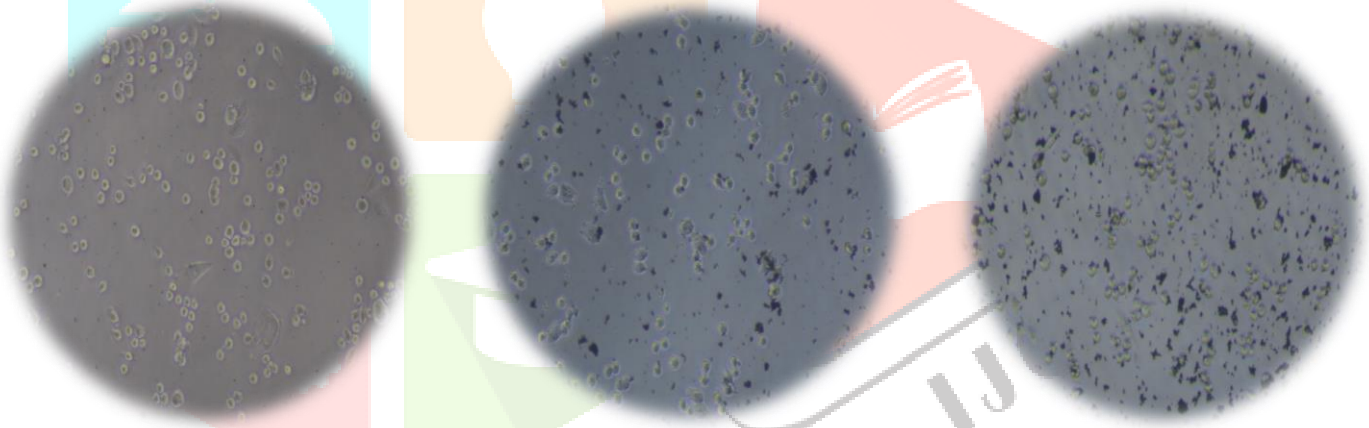
Particle size of *Satva Bhasma*



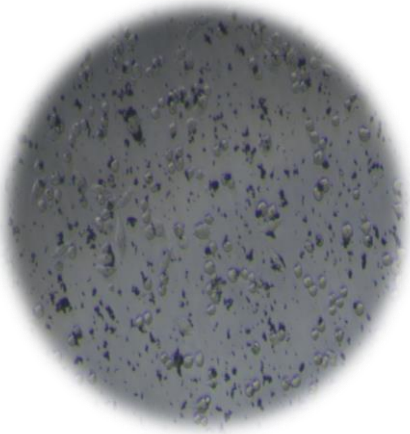
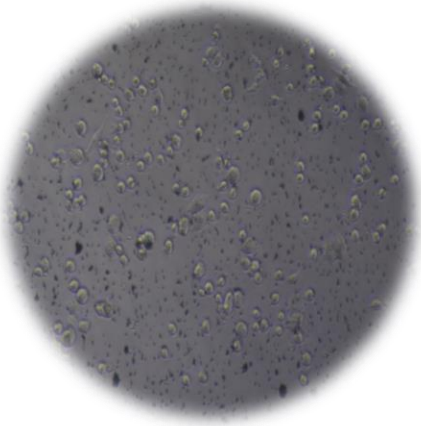
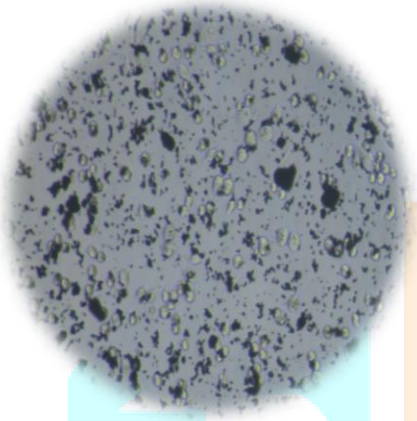


SEM-EDX of *Swarna Makshika Satva &Satva Bhasma*

- **MICROSCOPIC IMAGES OF SWARNA MAKSHIKA SATVA BHASMA'S ACTION AT DIFFERENT CONCENTRATIONS IN MIA PaCa-2 cell line.**



At 15.6 ug/ml Concentration **At 31.2 ug/ml Concentration** **At 62.5 ug/ml Concentration**

At 125 ug/ml Concentration **At 250 ug/ml Concentration** **At 500 ug/ml Concentration**

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