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Immunomodulatory effects of *Swarnamakshika Bhasma* : A Experimental Study

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ABSTRACT

The present day lifestyle and food habits have increased the production of free radicals. These cytotoxic free radicals not only raise the oxidative stress but also play an important role in the immune-system dysfunction due to which the mankind is prone to various major ailments and it is now proved that diseases like *Prameha, Pandu, Vatavyadhi* etc. are free radical mediated ones. To tackle these free radicals our body needs antioxidants. An antioxidant is a molecule which is capable of inhibiting the oxidation of other molecules. Oxidation reactions can produce free radicals which in turn start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions. Many herbals drugs and compound herbal preparations have been screened for their antioxidant and immuno-modulatory properties but still there is a need for effective antioxidants. This dearth and also the fact that *Swarnamakshika* is being used in treating many of the free radical mediated diseases prompted us to take the present study which aims to validate the immuno-modulatory effect of *Swarnamakshika Bhasma* scientifically and explain its probable mode of action at the cellular level.

Key words: *Makshika, Shodhana, Marana, Amritikarana, Immunomodulatory.*

INTRODUCTION

Immunomodulators are biological entities exerts effect by improving defense mechanism against disease and that have the capability to suppress an immune response. Immunomodulation is a process which can alter the immune system of an organism by interfering with its functions and results in an enhancement of immune reactions either in immunostimulent or immunosuppressant. The concept of '*Rasayana*' is based on related principles.[1] *Rasayana*, listed as a class in the texts of traditional Ayurvedic medicine literature, consists of a number of drugs reputed to promote physical and mental health, improve the ojus and defense mechanisms of the body and enhance longevity. In Ayurveda particularly *Rasayana* is recommended for Immune system. Many herbs, minerals, metals, gem stones and some poisonous drugs also having property of *Rasayana*. In my studies proved that *Swarnamakshika* is having immunomodulatory and anti-oxidant property effect, Keeping this in mind this a experimental work is started.

Free radicals and other reactive oxygen species are derived either from normal essential metabolic processes in the human body (endogenous) or from external sources (exogenous). Free radical formation occurs continuously in the cells as a consequence of both enzymatic and non-enzymatic reactions. Enzymatic reactions which serve as sources of free

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ISSN: 2456-3110 ORIGINAL ARTICLE Sept-Oct 2019

radicals include those involved in the respiratory chain, in phagocytosis, in prostaglandin synthesis and in the cytochrome P450 system. Free radicals also arise in non-enzymatic reactions of oxygen with organic compounds as well as those initiated by ionizing radiations. An immuno-modulator is a substance which has an effect on the immune system. There are two types of effects - immunostimulation and immunosuppression. Most drugs however do not have effects on only one receptor, so an immuno-modulator may be at the same time an immunosuppressant and an immuno-stimulant, on different targets within the immune system.[2]

Materials and Methods

Pharmaceutical study of *Swarnamakshika Bhasma* is designed as below

- Collection of raw drugs.
- *Swarnamakshika Shodhana*.
- Preparation of *Swarnamakshika Bhasma*s by using *Varaha Puta*.

Collection of raw drugs

The Raw *Swarnamakshika* was collected from (hinustan copper ltd.) Khetri Copper Complex Khetrinagar, Rajastan. It was having all *Grahya Lakshanas*[3] told in the classics. The Raw drug was confirmed by Quantitative analysis for Copper, Iron and Sulphur using Atomic Absorption Spectrophotometer study.

Swarnamakshika Shodhana[4]

Powder of *Swarnamakshika* was taken in an iron vessel. 1000ml of *Erand Taila* was added to it and mixed properly. Then this iron vessel was kept on a gas stove for heating *Agni* is in *Tivragni*. The mixture was then continuously stirred with the help of a small darvi throughout the procedure. Heating was stopped when the bottom of an iron vessel attained red color and all sulphur fumes stoped. Then the mixture was washed thoroughly with the hot water for two times and kept spread for drying on a clean cloth.

Table 1: Showing the colour change of *Swarnamakshika Churna* during *Bharjana*.

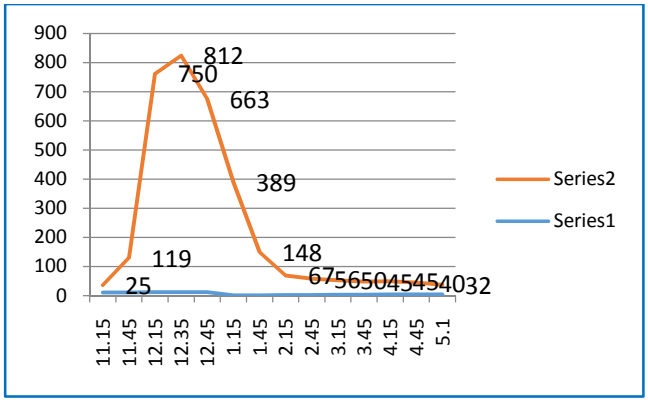
Before *Shodhana*	Warm gray
After addition of *Eranda Taila*	Light gray
After 5min addition of *ErandaTaila*	Greenish gray
After 15 min of heating	Blackish gray
After 40 min of heating	Blackish gray red color

Preparation of *Swarnamakshika Bhasma* by using *Varaha Puta*[5]

- Finely powdered *Shudda Swarnamakshika* was taken in a *Khalva Yantra.*
- Then equal quantity of *Shudda Gandhaka* was added and triturated together till they become homogenous.
- To this mixture 100ml of *Nimbu Swarasa* was added triturated well till it becomes semisolid consistency.
- The paste were made into shape of *Chakrikas* weighing 25gm and 8cm uniformly and kept for drying.
- In first Sharava the *Swarnamakshika Chakrikas* were arranged separately leaving little space to avoid overlapping.
- Over this *Sharava*, another *Sharava* was placed invertedly. Facing mouth of two *Sharavas* in contact, without leaving any space at the juncture.
- *Sandhi Bhandana* was done with the help of a cloth smeared with *Multani mitti*.
- Seven Layers of multani mitti were applied, allowed to dry.
- Completely dried *Samputa* was subjected to *Varaha Puta*.
- Measurement of *Varaha Puta*: 16” x 16” x 16” Angulas (lxbxd)
- *Vanopalas* used: 12½ kgs

ISSN: 2456-3110 ORIGINAL ARTICLE Sept-Oct 2019

Graph 1: Showing the time & temp during *Varaha Puta*.



***Amritikarana* of *Swarnamakshika*[6]**

Swarnamakshika Bhasma with equal quantity of *Panchamrita* is taken in a *Lohapatra* and this mixture is covered with *Sharava* and subjected to *Paka*, till it becomes *Nirdhuma*. After *Swangasheeta* this mixture is collected and subjected to *Bhavana* with *Triphala Kwatha, Chakrikas* are prepared and subjected to five *Varahaputas.*

ANALYTICAL STUDY

The metallic and mineral preparation of Ayurvedic pharmacopoeia should be analyzed for physical and chemical properties to confirm the genuinety and safety before administration to the patients. Hence the main aim of the study was to scientifically validate the raw drugs used to prepare the *Bhasma* and to ensure good quality of the prepared *Swaranamakshika Bhasma* by classical and modern analytical parameters. With this intention the raw drugs, intermediate products and the final product were subjected for analysis by employing various suitable parameters and methods. In the present study sample is collected from Khetri Copper Mine (Hindustan copper Ltd) Rajasthan.

Particle size analysis of for *Swarnamakshika Bhasma* was done at "Indian Institute of Technology" Bombay

Screening of Immunomodulatory effect of *Sawrnamakshika Bhasma*[7]

Materials Required

- Drug: *Swarnamakshika Bhasma.*
- Blood samples: Three (A, B, and C,)
- Equipments and Glassware: Micropipette, Water bath, Incubator, Centrifuge, Weighing Machine, Top pan balance, Measuring cylinders, Test Tubes and Slides, Light Microscope (having oil-immersion objective)

A. Nitro Blue Tetrazolium Test

The cells are exposed to the yellow dye Nitroblue tetrazolium (NBT). Unstimulated neutrophils do not ingest this dye but if the cells are stimulated to phagocytic activity, they take the dye into phagosomes and intracellular reduction of the dye converts it to an insoluble, blue crystalline form (formazan crystals).These blue crystals are visible in the light microscope and can be counted. The NBT test gives information about phagocytic function, since the dye is not taken into cells except by phagocytosis.

Preparation of Chemicals and Reagents

- E. coli Endotoxin Standard: 20 ml of broth from each of 5 strains of E.coli was taken and boiled on water bath for 2 hrs. Then centrifuged at 2000 rpm for 30 min and pooled the supernatant to store it as 1 ml aliquots at -20^0C.
- Nitro blue tetrazolium (NBT): 30 mg of NBT powder added in 10 ml of sucrose solution (Sucrose powder 35 mg + 10 ml D/W added) and mixed well.
- Minimum Essential Medium (MEM): 1.60 gm of MEM powder was dissolved in 100 ml of D/W.

Preparation of test sample

Stock solution for immunomodulatory study was prepared by dissolving *Swarnamakshika Bhasma* in Dimethyl Formamide (DMF) and diluted with Normal saline to obtain required concentrations.

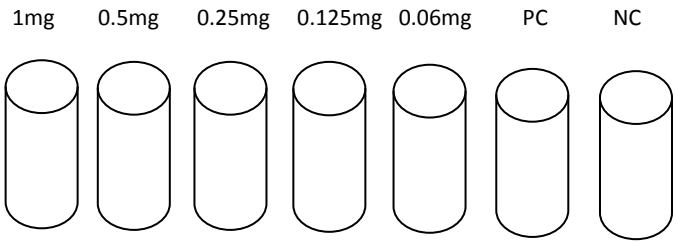
Estimation of NBT positive cells

- Five healthy blood samples were taken viz., A, B, and C,.
- A suspension of leucocytes ($5x10^6$ /ml) was prepared in 0.5 ml Phosphate buffer solution (PBS) in seven test tubes of each sample.

ISSN: 2456-3110 ORIGINAL ARTICLE Sept-Oct 2019

- 0.1 ml endotoxin-activated plasma (standard) was added to one tube (PC).
- 0.2ml of freshly prepared 0.15% NBT solution was added.
- Incubatedat 37°C for 20 min.
- Then gently centrifuged at 400 rpm for 3-4 min.
- The supernatant was discarded.
- One drop of PBS was added and gently re-suspended the cells in the small volume of fluid at the bottom of the tube.
- A thin film was prepared with a drop of this fluid on a microscope slide.
- Slides were dried for 10-15 min. Methanol fixation was carried out and again slides were kept for drying. Then stained in Giemsa stain for 15 min and washed under tap water. After complete drying the slides were observed under light microscope with oil-immersion objective.
- Using an oil immersion objective, 300 neutrophils were counted and the % of NBT positive cells containing blue deposits determined.

Fig. 2: Filling of test tubes for NBT Test *Swarnamakshika Bhasma* of Sample A



Similarly it is done for Sample B, and C.

Table 4: Filling of each test tube in NBT

Ingredients for NBT Test	1mg Suspension	0.5mg Suspension	0.25 mg Suspension	0.125 mg Suspension	0.06 mg Suspension	PC(Positive control)	NC(Normal control)
MEM	150µl	200µl	225µl	237.5 µl	245µl	200µl	250µl
NBT	50µl	50µl	50µl	50µl	50µl	50µl	50µl
DRUG	100µl	50µl	25µl	12.5 µl	6.25 µl	-	-
BLOOD	100µl	100µl	100µl	100µl	100µl	100µl	100µl
ENDO-TOXIN	-	-	-	-	-	50µl	-

B. Phagocytosis and Candidacidal Assay

It is the twin method which can be performed at the same time for phagocytosis and for Candidacidal assay. The cells are exposed to the candida albicans suspension. If the leucocytes are stimulated to phagocytic activity, the majority of candida cells will be engulfed by them.The leucocytes containing candida cells are clearly visible under light microscope which can be counted and MPN (Mean Particle Number) can be calculated.

In Candidacidal assay, Sodium deoxycholate and Methylene Blue are added to the same cells containing candida albicans. Sodium deoxycholate lyses the leucocytes but do not damage candida cells. Methylene blue is used for staining of dead candida cells. Using an improved Neabauer counting chamber the proportion of dead cells i.e. those which have taken up the methylene blue can be determined.

Preparation of chemicals and reagents

a) Minimum Essential Medium (MEM): was prepared same as in the NBT test.

b) Sodium deoxicholate 2.5% in distilled water, pH = 8.7

c) Methylene Blue solution: 0.01% in 0.15 M NaCl

Isolating Neutrophils by Dextran sedimentation method

Materials

i) Dextran solution – 6% in 0.15 M NaCl

ii) MEM – As tissue culture medium

ISSN: 2456-3110 ORIGINAL ARTICLE Sept-Oct 2019

iii) Preservative free heparin – 15U/ml blood

Method

Heparinized blood sample, 3 ml was diluted with 3 ml of MEM. Diluted blood was then mixed with 1.5 ml of 6% Dextran solution carefully which causes the red cells to sediment rapidly leaving an upper layer of leucocyte-rich plasma. This was kept at room temperature for 45 min without disturbing. The supernatant was removed into 3 to 4 centrifuge tubes.

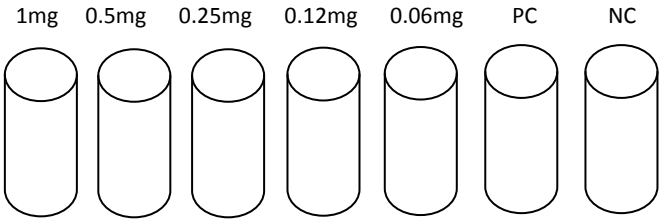
These tubes were then centrifuged at 500 rpm for 10 min. The supernatant was discarded and the cells were carefully flicked up. A small volume of PBS was used to wash the contents and centrifuged again. Above step was repeated for 2-3 times after which the cells were flicked up and finally 850µl of MEM was added to make the volume up to 1 ml.

Candida albicans suspension

Candida albicans were grown on glucose peptone agar. A culture suspension of 24 hour old was prepared and used for test.

Phagocytosis and Candidacidal Assay

Fig 3: Filling of test tubes for Phagocytosis and candidacidal assay SM B



Blood Sample A

Similarly done for sample B and C

Table 5: Filling of test tubes for Phagocytosis and Candidacidal Assay

Ingredients for Test	1mg Suspension	0.5mg Suspension	0.25 mg Suspension	0.125 mg Suspension	0.06 mg Suspension	PC(Positive control)	NC(Normal control)
MEM	100µl	150µl	175µl	188µl	195µl	100µ	200µ
CANDIDA	100µl	100µl	100µl	100µl	100µl	100µl	100µl
DRUG	100µl	50µl	25µl	12.5 µl	6.25 µl	-	-
WBC's	100µl	100µl	100µl	100µl	100µl	100µl	100µl
SERUM	-	-	-	-	-	100µl	-

- All the test tubes were kept in an incubator for 30 min at 37°C. Then 0.1 ml from each test tube was taken and smear prepared on glass slides for phagocytosis assay.
- Further all test tubes were incubated in an incubator for another 30 minutes at 37°C for Candidacidal assay. Meanwhile, for phagocytosis assay, methanol fixation and Giemsa staining were carried out and slides were kept for drying.
- After 30 min, 250 µl of 2.5% Sodium deoxycholate and 1 ml of Methylene blue indicator were added to each tube. Centrifugation was done for 10 min at 1000 rpm.
- Supernatant liquid was discarded and only the sediment part was collected. One drop from each test tube was taken on separate slides. Cover slips were placed over that drops and each slide was then observed under light microscope for dead candida cells and counted at least 300 cells per slide using Neubauer's chamber.

C. Neutrophil Locomotion And Chemotaxis Test:

When the cells are placed in a gradient of chemo attractant, the cells change their shape as they orient and migrate in unison towards the source of stimulus, a process called as "chemotaxis". Most of the neutrophil locomotion assesses the behavior of a population of cells moving through cellulose nitrate filters or under agarose. The cells are allowed to move a set time period then fixed, stained and assessed.

Preparation of chemicals and reagents

a) 0.24 gm of agarose was dissolved in 10 ml of distilled water by heating on water bath for 10-15 min and then cooled.

ISSN: 2456-3110 ORIGINAL ARTICLE Sept-Oct 2019

b) Supplemented MEM

- 3 ml MEM
- 3 ml heat inactivated pooled human serum
- 250 µl of 7.5% sodium bicarbonate
- 8 ml of sterile distilled water

c) 10^{-8} M. Fm-leu-phe (As known chemo attractant)

d) Staining reagents per plate

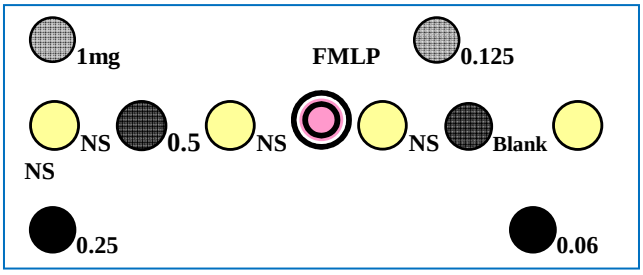
- 3-5 ml of methanol
- 3-5 ml of formaline
- Giemsa stain

➢ **Preparation of agarose culture plates:** 10 ml of pre-warmed supplemented MEM was added to 10 ml of agarose solution and around 5 ml of this mixture was added to each culture plate. The medium was then allowed to cool and solidify. After solidifying, appropriate wells in agarose were made.

➢ **Arrangement of wells in agarose plates:** Using Pasteur pipette, wells were prepared carefully measuring 3 mm in the diameter and spaced apart.

➢ **Neutrophil locomotion and chemotaxis test:**Three samples of neutrophil suspensions from blood were taken viz A, B, and C. The method for neutrophil isolation was carried out according to previous method i.e. phagocytosis and candidacidal assay. Later the wells in agarose were filled.

Fig. 4: Filling of wells for Chemotaxis Test



NS: Neutrophil suspension

Same design was followed for all the samples A, B, C, D and E.

- 0.01 ml of Fm-leu-phe was added to the most centrally located well of the slides. 0.01 ml of Neutrophil suspension was added to each of the blank well as shown in the above figure. Lastly, 0.01 ml of 1mg, 0.5mg. 0.25mg, 0.125mg and 0.06mg suspension of *Swarnamakshika Bhasma* were added to the shaded well in three direction of one side of the slide. Same was repeated on the other end of the slides.
- After putting all the suspension, slides were kept for charging i.e. kept in an incubator at 37^0C for 2 hrs. Then the slides were flooded in methanol for 30 min. The slides were kept flooding in formaline for 30 min. Agarose gel was removed carefully from slides. Giemsa staining was carried out and slides were kept for drying purpose. After complete drying, slides were observed under light microscope. A distance traveled by the cells (distance of cell's migration) was noted.

RESULTS

Immunomodulatory effect of *Swarnamakshika* Bhasma

a) NBT ASSAY:

Table 6: Effect of SMB on NBT parameter (Stimulated cells in %)

Blood Sample	Stimulated cells (%)						
	1 mg	0.5 mg	0.25 mg	0.125 mg	0.06 mg	PC	NC
Sample A	48	28	15	15	15	70	16
Sample B	32	20	20	16	16	66	19
Sample C	42	29	20	17	17	72	16
Mean±SD	40.67 ± 4.667	25.67± 284	18.33 ± 1.66	16.00 ± 0.577	16.00 ± 0.57	69.33 ± 1.764	17.00 ± 1.000

ISSN: 2456-3110 ORIGINAL ARTICLE Sept-Oct 2019

Table 7 : Comparison of doses of *Swaranamakshika Bhasma* with PC group

Mean score of PC	Different doses		Mean difference	p value	Significantly different
	Dose (mg)	Mean ± SD			
69.33 ± 1.764	0.06	16.00 ± 0.57	53.33 ± 1.8	< 0.0001****	Yes
	0.125	16.00 ± 0.577	53.33 ± 1.8	< 0.0001****	Yes
	0.25	18.33 ± 1.66	51.00 ± 2.4	< 0.0001****	Yes
	0.5	25.67± 284	43.67 ± 3.35	0.0002***	Yes
	1.0	40.67 ± 4.667	28.67 ± 4.98	0.0045**	Yes

Values expressed as Mean±SD; n=3 , ***p<0.001

Data were analyzed by one way ANOVA followed by Tukeys multiple comparison test. Positive control group showed significant (p<0.001) increase in the percentage of NBT- stimulated cells when compared to different doses of *Swarnamakshika Bhasma*.

Table 8 : Comparison of doses of *Swarnamakshika* with NC

Mean score of NC	Different doses		Mean difference	p value	Significantly different
	Dose (mg)	Mean ± SD			
17.00 ± 1.000	0.06	16.00 ± 0.57	1.000 ± 1.155	0.4353	No
	0.125	16.00 ± 0.577	1.000 ± 1.155	0.4353	No
	0.25	18.33 ± 1.66	-1.333 ± 1.944	0.5304	No
	0.5	25.67±284	-8.667 ± 3.018	0.0454*	Yes
	1.0	40.67 ± 4.667	-23.67 ± 4.773	0.0077**	Yes

Values expressed as Mean±SD, ***p<0.001 , *p<0.05

Data were analyzed by one way ANOVA followed by Tukeys multiple comparison test. The doses of *Swarnamakshika Bhasma* 1.0mg and 0.5mg showed significant (p<0.0077) increase in the percentage of NBT-stimulated cells except *Swarnamakshika* 0.25 mg, 0.125mg and 0.06mg (p<0.05) when compared to NC group.

B) Phagocytosis Assay

Table 9 : Effect of SMB on Phagocytosis (Mean Particle Number)

Blood Sample	Candida engulfed (MPN)						
	1 mg	0.5 mg	0.25 mg	0.125 mg	0.06 mg	PC	NC
Sample A	4	4	3	3	3	4	3
Sample B	4	4	4	4	3	5	3
Sample C	5	4	4	3	3	4	3
Mean ± SD	4.333 ± 0.333	4.000 ± 0.0	3.667 ± 0.33	3.333 ± 0.33	3.000 ± 0.0	4.333 ± 0.333	3.000 ± 0.0

Table 10 : Comparison of doses of *Swarnamakshika* with PC

Mean score of PC	Different doses		Mean difference	p value	Significantly different
	Dose (mg)	Mean ± SD			
4.333 ± 0.333	0.06	3.000 ± 0.0	1.333 ± 0.33	0.0161*	Yes
	0.125	3.333 ± 0.33	1.000 ± 0.47	0.1012	No
	0.25	3.667 ± 0.33	0.6667 ± 0.47	0.2302	No
	0.5	4.000 ± 0.0	0.3333 ± 0.33	0.3739	No
	1.0	4.333 ± 0.333	0.0 ± 0.4714	> 0.9999	No

Values expressed as Mean±SD, n=5, *p<0.05, ***p<0.001, NS= non-significant.

Data were analyzed by one way ANOVA followed by Tukeys multiple comparison test. Positive control

ISSN: 2456-3110 ORIGINAL ARTICLE Sept-Oct 2019

group showed significant (p<0.001) phagocytic activity at the dose of 0.6mg when compared to different doses of *Swarnamakshikabhasma*.

Table 11 : Comparison of doses of *Swarnamakshika* with NC

Mean score of NC	Different doses		Mean difference	p value	Significantly different
	Dose (mg)	Mean ± SD			
3.000 ± 0.0	0.06	3.000 ± 0.0	***	***	***
	0.125	3.333 ± 0.33	-0.3333 ± 0.3	0.3739	No
	0.25	3.667 ± 0.33	-0.6667 ± 0.3	0.1161	No
	0.5	4.000 ± 0.0	***	***	***
	1.0	4.333 ± 0.333	-1.333 ± 0.33	0.0161	Yes*

Note: *** indicates All the values in one of the columns are identical. Cannot calculate unpaired 't'test In case of *Swarnamakshika* 1 mg, there was significant (p<0.001) increase in the phagocytic activity when compared to Negative control group (NC).

Candidacidal Assay

Table 12: Effect of SMB on Candidacidal assay (Dead candida cells in %)

Blood Sample	Dead Candida cells (%)						
	1 mg	0.5 mg	0.25 mg	0.125 mg	0.06 mg	PC	NC
Sample A	28	15	12	12	12	36	12
Sample B	39	18	15	15	15	40	15
Sample C	28	18	16	16	16	37	18
Mean± SD	31.67 ± 3.6	17.00 ± 1.0	14.33 ± 1.2	14.33 ± 1.2	14.33 ± 1.2	37.67 ± 1.2	15.00 ± 1.73

Table 13: Comparison of doses of *Swarnamakshika* with PC

Mean score of PC	Different doses		Mean difference	p value	Significantly different?
	Dose (mg)	Mean ± SD			
37.67 ± 1.2	0.06	14.33 ± 1.2	23.33 ± 1.7	0.0002***	Yes
	0.125	14.33 ± 1.2	23.33 ± 1.7	0.0002***	Yes
	0.25	14.33 ± 1.2	23.33 ± 1.7	0.0002***	Yes
	0.5	17.00 ± 1.0	20.67 ± 1.5	0.0002***	Yes
	1.0	31.67 ± 3.6	6.000 ± 3.8	0.1949	No

Values expressed as Mean±SD, n=5, ***p<0.001, NS= non-significant.

Data were analyzed by one way ANOVA followed by Tukeys multiple comparison test. Positive control showed significant (p<0.001) increase in the dead candida cells when compared to *Swarnamakshika* 1.0 mg.

Table 14: Comparison of doses of *Swarnamakshika* with NC

Mean score of NC	Different doses		Mean difference	p value	Significantly different?
	Dose (mg)	Mean ± SD			
15.00 ± 1.73	0.06	14.33 ± 1.2	0.6667 ± 2.1	0.7676	No
	0.125	14.33 ± 1.2	0.6667 ± 2.1	0.7676	No
	0.25	14.33 ± 1.2	0.6667 ± 2.1	0.7676	No
	0.5	17.00 ± 1.0	-2.000 ± 2.00	0.3739	No
	1.0	31.67 ± 3.6	-16.67 ± 4.0	0.0147*	Yes

Doses of *Swarnamakshika Bhasma* 1mg showed significant (***p<0.001) increase in the dead candida

ISSN: 2456-3110 ORIGINAL ARTICLE Sept-Oct 2019

cells except other doses (**p<0.01) when compared to negative control group.

Neutrophil locomotion and Chemotaxis Assay

Table 15: Results of Neutrophil Locomotion and Chemotaxis test (In mm)

Blood Sample	Movement of neutrophils						
	1 mg	0.5 mg	0.25 mg	0.125 mg	0.06 mg	PC	NC
Sample A	1.8	1.8	1.2	0.8	0.8	2	0.5
Sample B	1.9	1.8	1.4	1.0	0.6	2.2	0.5
Sample C	2	1.7	1.2	1.0	0.9	2.1	0.5
Mean±SD	1.900 ± 0.05	1.767 ± 0.03	1.267 ± 0.066	0.9333 ± 0.06	0.7667 ± 0.088	2.100 ± 0.057	0.5000 ± 0.0

Table 16 : Comparison of doses of *Swarnamakshika* with PC

Mean score of PC	Different doses		Mean difference	p value	Significantly different?
	Dose (mg)	Mean ± SD			
2.100 ± 0.057	0.06	0.7667 ± 0.08	1.333 ± 0.10	0.0002***	Yes
	0.125	0.9333 ± 0.06	1.167 ± 0.08	0.0002***	Yes
	0.25	1.267 ± 0.066	0.8333 ± 0.08	0.0007***	Yes
	0.5	1.767 ± 0.03	0.3333 ± 0.06	0.0075**	Yes
	1.0	1.900 ± 0.05	0.2000 ± 0.08	0.0705	No
Values expressed as Mean±SD, n=5, ***p<0.001					

Data were analyzed by one way ANOVA followed by Tukeys multiple comparison test. Positive control showed significant (***p<0.001) increase in the neutrophil and chemo tactic activity when compared to different doses of *Swarnamakshika* 1mg

Table 17: Comparison of doses of *Swarnamakshika* with NC

Mean score of NC	Different doses		Mean difference	p value	Significantly different
	Dose (mg)	Mean ± SD			
0.5000 ± 0.0	0.06	0.7667 ± 0.08	-0.2667 ±0.08	0.0390*	Yes
	0.125	0.9333 ± 0.06	-0.4333 ± 0.06	0.0029**	Yes
	0.25	1.267 ± 0.066	-0.7667 ± 0.06	0.0003***	Yes
	0.5	1.767 ± 0.03	-1.267 ± 0.033	< 0.0001****	Yes
	1.0	1.900 ± 0.05	-1.400 ± 0.057	< 0.0001****	Yes

Swarnamakshika showed significant difference in (***p<0.001)neutrophil and chemotactic activity at the dose of 1mg and 0.5mg when compared to negative control.

DISCUSSION

In present work, *Swarnamakshika Bhasma* was subjected to assess Immuno-modulatory activity in three different Samples for which parameters like NBT test, phagocytosis and Candidacidal assay and Neutrophil locomotion and Chemo tactic assay were evaluated.Intracellular reduction of NBT of *Swarnamakshika* has significantly increased the dye to Formosan crystals by neutrophils confirming the intracellular killing property when compared to Negative Control. *Swarnamakshika Bhasma* by NBT test confirms the stimulating effect on cells at the dose of 1mg. This motivated immunity further confirms the phagocytic function of neutrophils by engulfing the candida cells and also confirms the candidacidal effect significantly which was near to PC

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value.The movement of neutrophils towards the foreign body by *Swarnamakshika Bhasma* is highly significantly increase in all the doses, which is most important step in phagocytosis process.

CONCLUSION

Shodhana by *Bharjana* process in *Eranda Taila, Bhavana* with *Jambhira Swarasa* and *Marana* by 12 *Varaha Puta* were sufficient to obtain *Swarnamakshika* Bhasma which passed all the *Bhasma Parikshas*. Increase in the NBT assay, Phagocytosis and chemotaxis represents good Immuno-modulatory effect of *Swarnamakshika Bhasma* at the dose of 1mg. It suggests that in higher doses it may act as cytotoxic agent but acts as immunostimulant when applied in smaller doses. Immuno-modulatory study also revealed that *Swarnamakshika Bhasma* 1mg showed statistically significant results. By the present study, we can attribute the *Acharya Charaka's* basic concept; "प्राकृतस्तुबलंश्लेष्मा...|" which denotes the *Vyadhikshamata*. According to Acharya Sushruta, "ओजस्तुसोमात्मकं....|". Here *Swarnamakshika Bhasma* having above said qualities will increase the *Ojas* and also increase the *Prakrita Kapha* which will serve the purpose of *Balya* and *Rasayana*. The outcomes of whole present study can be attributed to the above said concept of *Balya*, *Rasayana* etc.

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