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Immuno-Modulatory activity of Mukta Bhasma (Calx of Cultured Pearls)

Research Article

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Abstract

Objective: Immune activation is an effective as well as protective approach against emerging infectious diseases. To screen for in-vitro immuno-modulatory activity of *Mukta Bhasma* (Calx of cultured Pearl) on human neutrophils. Methods: *Mukta Bhasma (MB)* was subjected to In-vitro screening to assess its Immunomodulatory effect using the Nitroblue Tetrazolium (NBT) assay, Phagocytosis & Candidacidal assay, Neutrophil locomotion and Chemotaxis test at five different doses of test drug (1mg, 0.5mg, 0.25mg, 0.125mg, 0.06mg). Results: *Mukta bhasma* has shown significant ($p<0.001$) increase in the % of NBT- stimulated cells and candida cells at 0.06mg. MB 0.125mg and MB 0.25 mg showed significant increase ($p<0.01$) in the candida cells when compared to MB 1.0mg. MB 0.06mg shows significant ($p<0.001$) increase in the phagocytic activity when compared to other doses of MB. MB showed significant ($p<0.001$) neutrophil and chemotactic activity at the dose of 0.06 mg and 0.125 mg. *Mukta bhasma* has shown significant($p<0.001$) increase in all the parameters at the dose of 0.06 mg when compared to other doses of MB. Conclusion: The results were self-conclusive and indicated that *Mukta Bhasma* is immune- potent and showed immuno-modulatory effect at lower doses. Thus, the study revalidates the reference of *Mukta Bhasma* as a *Rasayan* and hence also establishing it as an Immunomodulator.

Keywords: Immuno-modulatory activity, Mukta Bhasma, Rasayana, NBT assay & Phagocytosis, Candidacidal assay, Chemotaxis.

Introduction

Pearl/*Mukta* is a highly revered substance known for its potent therapeutic properties. Mukta bhasma (Calx of Cultured Pearl) is synthesized by trituration and calcination of purified Pearls classically. It is a well-known drug to promote strength & intellect, enhances semen production, powerful cardiac tonic, antitoxic and mood elevator (1). Mukta bhasma possessess significant gastroprotective activity in lower doses of therapeutic range by modulating scavenging of free radicals (2). Recent studies have scientifically validated its role in calcium absorption, and even in enhancing the effectiveness of antibiotics (3,4,5)

Immunotherapy is an upcoming clinical branch practiced during chemotherapy, in auto-immune & immune-compromised conditions (6). Rasayana herbs help in improving immunogenicity and work as potential immunomodulators (7). Thus the immunotherapy (Rasayana therapy) is not only beneficial in maintenance of health but also helpful in therapeutic management of disease state when used as a naimithika rasayana in defined dose & form.

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Autoimmune disorders arise when physiological tolerance to self antigens is lost. Although several mechanisms may be involved in this pathogenic process, dysregulation of T-cell and B-cell activation and of pathways leading to inflammation are logical candidates. Susceptibility to autoimmune diseases has been associated with multiple factors including genetics, epigenetics, and the environment.

Substances known as immunomodulators are used to alter immune system components (8). Numerous inflammatory illnesses have been treated using chemical immunomodulators such as hydrocortisone, dexamethasone, and prednisone that are readily available in the market. Sadly, the majority of these over-the-counter medications have multiple adverse effects. Consequently, safer and more potent medications are needed as substitutes. Natural goods continue to be a significant source of innovative, safe medications.

Based on results of our earlier research works, which focused on in-vivo screening of antioxidant activity of *Mukta bhasma*, showed good antioxidant activity at the dose of 200mg/kg in albino rats (9). With this view, study was undertaken to evaluate the immunomodulatory effect of *Mukta Bhasma* invitro.

Materials and Methods

Preparation of Test drug (MB)

Mukta bhasma was prepared according to the reference text *Rasa tarangini*(10). *Mukta* (Pearl) were

first cleaned with hot water. Cleaned *Mukta* were immersed in the juice of leaves of *Sesbania sesban (L)Merr (Jayanti*) and boiled (*swedana*) for three hours in specially prepared hanging sealed earthen pot (*Dola yantra*). The purified *Mukta* were grinded to powder form in a mortar and pestle, triturated with Cow's milk and made into round pellets. Then they were subjected to calcination in traditional furnace (*laghu puta*) which reached about mean temperature 700^0- 800^0C. This procedure is repeated for three more times to get proper calx (*bhasma*).

In-vitro* immuno-modulatory screening of *Mukta Bhasma:

Nitroblue-Tetrazolium Test (11)

Principle

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.

Procedure

Preparation of Chemicals and Reagents:

- E. coli Endotoxin Standard (Hi-Media Co.): 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.

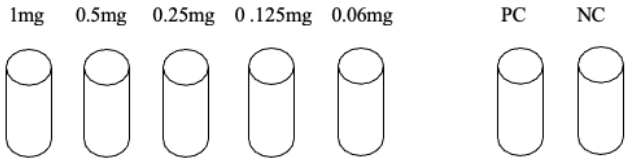
Preparation of Test sample

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

Estimation of NBT positive cells

Five blood samples from healthy individual were taken viz., A, B, C, D & E. Suspension of leucocytes ($5x10^6$ /ml) was prepared in 0.5 ml Phosphate buffer solution (PBS) in seven test tubes of each sample. 0.1 ml endotoxin-activated plasma (standard) was added to one tube (Positive Control). 0.2ml of freshly prepared 0.15% NBT solution was added. Incubated at 37^0C for 20 min. The sample was centrifuged gently for 3–4 minutes at 400 rpm. 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. Using an oil immersion objective, 300 neutrophils were counted and the % of NBT positive cells containing blue deposits determined. (Table No:1)

Fig. 1: Filling of each test tube for NBT Test Sample A M.Bh.



Similarly, it is done for Sample B, C, D and E.

Table 1: Filling of each test tube in NBT

Ingredients for NBT Test	1mg Suspension	0.5 mg Suspension	0.25 mg Suspension	0.125mg Suspension	0.06mg Suspension	PC (Positive control)	NC (Normal control)
MEM	150ml	200ml	225ml	237.5ml	245ml	200ml	250ml
NBT	50ml	50ml	50ml	50ml	50ml	50ml	50ml
Drug	100ml	50ml	25ml	12.5ml	6.25ml	-	-
Blood	100ml	100ml	100ml	100ml	100ml	100ml	100ml
Endotoxin	-	-	-	-	-	50ml	-

Phagocytosis and Candidacidal Assay (11)

Principle

It is the twin method which can be performed at the same time for Phagocytosis and for Candidacidal assay. The suspension of Candida albicans is exposed to the cells. The most of candida cells will be ingested by leucocytes if they are activated to engage in phagocytic activity. Under a light microscope, the leucocytes harboring the candida cells are easily visible, allowing for the counting and calculation of the MPN (Mean Particle Number).

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 Neubaer counting chamber, the proportion of

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dead cells i.e.those which have taken up the methylene blue can be determined.

Procedure

Preparation of chemicals and reagents

- Minimum Essential Medium (MEM): was prepared same as in the NBT test.
- Sodium deoxicholate 2.5% in distilled water, pH = 8.7
- Methylene Blue solution: 0.01% in 0.15 M NaCl

Isolating Neutrophils by Dextran sedimentation method

Materials

- Dextran solution – 6% in 0.15 M NaCl
- MEM – As tissue culture medium
- 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.

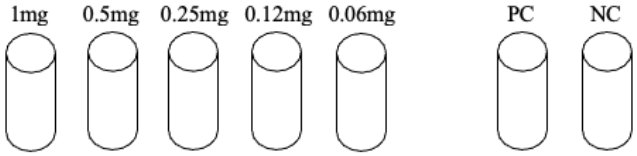
Candida albicans suspension: Candida albicans were grown on glucose peptone agar. A culture suspension of 24 hour old was prepared and used for test.

Phagocytosis & Candidacidal Assay

- All the test tubes were kept in an incubator for 30 min at 37^{0}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^{0}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 ml 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.

Figure 2: Filling of test tubes for Phagocytosis and Candidacidal assay

Sample A M.B.



Similarly done for sample B, C, D and E.

Table 2: Filling of test tubes for Phagocytosis and Candidacidal Assay

Ingredients for Test	1mg Suspension	0.5mg Suspension	0.25 mg Suspension	0.125mg Suspension	0.06mg Suspension	PC (Positive control)	NC (Normal control)
MEM	100ml	150ml	175ml	188ml	195ml	100ml	200ml
Candida	100ml	100ml	100ml	100ml	100ml	100ml	100ml
Drug	100ml	50ml	25ml	12.5ml	6.25ml	-	-
WBC's	100ml	100ml	100ml	100ml	100ml	100ml	100ml
Serum	-	-	-	-	-	100ml	-

Neutrophil locomotion and chemotaxis test(11)

Principle

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.

Procedure

Preparation of chemicals and reagents

- 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.
- 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

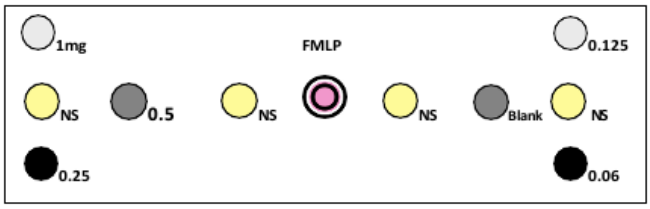
- 10^{-8} M. Fm-leu-phe (As known chemo attractant)
- Staining reagents per plate
  - 3-5 ml of methanol
  - 3-5 ml of formaline
  - Giemsa stain

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: Five samples of neutrophil suspensions from blood were taken viz A, B, C, D and E. 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.

Figure 3: 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 Mukta 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^{0}C for 2 hrs. Then the slides were flooded in methanol for 30 min. The slides were kept flooding in formalin 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

NBT Assay

Data was analyzed by One way ANOVA followed by Tukeys multiple comparison test. *Mukta bhasma* has shown significant ($p<0.001$) increase in the % of NBT-stimulated cells particularly in lower doses at 0.06mg. **(Table No. III & Table No.IV) (fig No 4).**

Table 3: Effect of *Mukta Bhasma* on NBT parameter (Stimulated cells in %)

Blood Sample	Stimulated cells (%)						
	1 mg	0.5 mg	0.25mg	0.125 mg	0.06mg	PC	NC
Sample A	40	38	30	51	63	70	28
Sample B	36	34	34	58	60	71	26
Sample C	30	30	32	50	58	72	26
Sample D	37	36	30	54	61	69	25
Sample E	36	34	31	52	60	70	27
Mean± SD	35.80± 3.633	34.40±2.966	31.40±1.673	53.0±3.162	60.40±1.817	70.40±1.140	26.40±1.140

Table IV: Comparison between the doses of MB

Name of group	Mean of both	Mean difference	p value
0.06 mg Vs 0.125 mg	60.40 vs 53.0***	6.862	<0.001
0.06 mg Vs 0.25 mg	60.40 vs 31.40***	26.89	<0.001
0.06 mg Vs 0.5 mg	60.40 vs 34.40***	24.11	<0.001
0.06 mg Vs 1.0 mg	60.40 vs 35.80***	22.81	<0.001
0.125 mg Vs 0.25 mg	53.0 vs 31.40***	20.03	<0.001
0.125 mg Vs 0.5 mg	53.0 vs 34.40***	17.25	<0.001
0.125 mg Vs 1.0 mg	53.0 vs 35.80***	15.95	<0.001
0.25 mg Vs 0.5 mg	31.40 vs 34.40	2.782	0.163 NS
0.25 mg Vs 1.0 mg	31.40 vs 35.80	4.080	0.163 NS
0.5 mg Vs 1.0 mg	34.40 vs 35.80	1.298	0.163 NS

***p<0.001 NS=Not significant

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Phagocytosis Assay

Table 5: Effect of *Mukta bhasma* on Phagocytosis (in 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	3	3	4.5	5	5	6	3
Sample B	3	4	4	5	5	6	4
Sample C	3	4	4	5	5	6	3
Sample D	3	3	3	4	5	5	3
Sample E	3	4	4	5	5	6	3
Mean±SD	3.0±0.0	3.6±0.54	3.9±0.54	4.8±0.44	5.0±0.0	5.8±0.44	3.2±0.44

Table 6: Comparison between the doses of Mukta Bhasma

Name of the group	Mean of both group	Mean difference	p value
0.06 mg Vs 0.125 mg	5.0 vs 4.8	0.20	NS
0.06 mg Vs 0.25 mg	5.0 vs 3.9**	1.10	<0.01
0.06 mg Vs 0.5 mg	5.0 vs 3.6***	1.40	<0.001
0.06 mg Vs 1.0 mg	5.0 vs 3.0***	2.00	<0.001
0.125 mg Vs 0.25 mg	4.8 vs 3.9*	0.90	<0.05
0.125 mg Vs 0.5 mg	4.8 vs 3.6**	1.20	<0.01
0.125 mg Vs 1.0 mg	4.8 vs 3.0***	1.80	<0.001
0.25 mg Vs 0.5 mg	3.9 vs 3.6	0.30	NS
0.25 mg Vs 1.0 mg	3.9 vs 3.0*	0.90	<0.05
0.5 mg Vs 1.0 mg	3.6 vs 3.0	0.60	NS
*p<0.05 **p<0.01 ***p<0.001			

MB 0.06mg shows significant (p<0.001) increase in the phagocytic activity when compared to MB 1.0 mg.

Candidacidal assay

Table 7: Effect of *Mukta bhasma* 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	21	30	36	40	37	36	16
Sample B	20	23	30	30	35	38	16
Sample C	34	34	34	37	35	36	16
Sample D	20	33	37	30	36	37	15
Sample E	30	31	35	36	38	36	16
Mean±SD	25.00±6.55	30.20±4.32	34.40±2.7	34.60±4.45	36.20±1.3	36.60±0.8	15.80±0.4

Table 8: Comparison between the doses of Mukta Bhasma

Name of the group	Mean of both group	Mean difference	p value
0.06 mg Vs 0.125 mg	36.20 vs 34.60	1.600	NS
0.06 mg Vs 0.25 mg	36.20 vs 34.40	1.800	NS
0.06 mg Vs 0.5 mg	36.20 vs 30.20	6.000	NS
0.06 mg Vs 1.0 mg	36.20 vs 25.00***	11.20	<0.001
0.125 mg Vs 0.25 mg	34.60 vs 34.40	0.200	NS
0.125 mg Vs 0.5 mg	34.60 vs 30.20	4.400	NS
0.125 mg Vs 1.0 mg	34.60 vs 25.00**	9.600	0.01
0.25 mg Vs 0.5 mg	34.40 vs 30.20	4.200	NS
0.25 mg Vs 1.0 mg	34.40 vs 25.00**	9.400	0.01
0.5 mg Vs 1.0 mg	30.20 vs 25.00	5.200	NS

MB 0.06 mg showed highly significant(***p<0.001) increase in the dead candida cells as compared to MB 1.0 mg. MB 0.125mg and MB 0.25 mg showed significant increase (**p<0.01) as compared to MB 1.0mg.

Neutrophil locomotion and chemotaxis assay

Table 9: 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	0.5	0.6	0.8	1.0	1.0	2.1	0.6
Sample B	0.6	0.7	1.0	1.0	1.2	2.0	0.5
Sample C	0.6	0.8	1.0	1.1	1.2	1.9	0.7
Sample D	0.7	0.6	0.8	1.0	1.1	2.1	0.5
Sample E	0.5	0.7	0.7	1.0	1.0	1.7	0.6
Mean±SD	0.58±0.08	0.68±0.08	0.86±0.13	1.02±0.04	1.10±0.10	1.96±0.16	0.58±0.08

Table 10: Comparison between the doses of MB

Name of the group	Mean of both group	Mean difference	p value
0.06 mg Vs 0.125 mg	1.10 vs 1.02	0.08000	NS
0.06 mg Vs 0.25 mg	1.10 vs 0.86*	0.2400	<0.05
0.06 mg Vs 0.5 mg	1.10 vs 0.68***	0.4200	<0.001
0.06 mg Vs 1.0 mg	1.10 vs 0.58***	0.5200	<0.001
0.125 mg Vs 0.25 mg	1.02 vs 0.86	0.1600	NS
0.125 mg Vs 0.5 mg	1.02 vs 0.68***	0.3400	<0.001
0.125 mg Vs 1.0 mg	1.02 vs 0.58***	0.4400	<0.001
0.25 mg Vs 0.5 mg	0.86 vs 0.68	0.1800	NS
0.25 mg Vs 1.0 mg	0.86 vs 0.58**	0.2800	<0.01
0.5 mg Vs 1.0 mg	0.68 vs 0.58	0.1000	NS

MB showed significant (p<0.001) neutrophil and chemotactic activity at the dose of 0.06 mg and 0.125 mg when compared to MB 0.5 mg and MB 1.0mg.

Figure 4: NBT graph

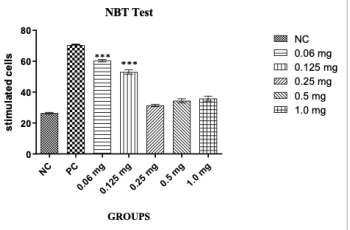


Figure 5: Phagocytosis graph

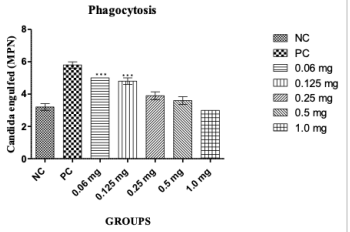


Figure 6: Candidacidal assay graph

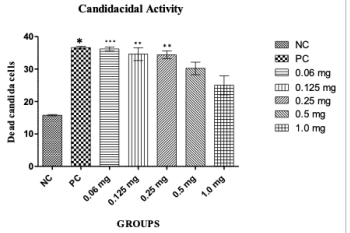
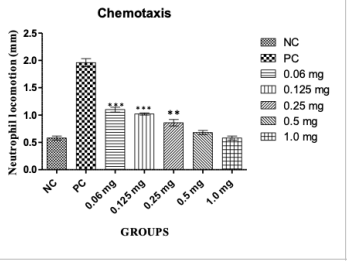


Figure 7: Chemotaxis & Neutrophil locomotion graph



Discussion

In present study, *Mukta Bhasma* was subjected to assess Immuno-modulatory activity in five different doses for which parameters like NBT test, Phagocytosis & Candidacidal assay and Neutrophil locomotion & Chemotactic assay were evaluated.

Mukta bhasma has shown significant (p<0.001) increase in the % of NBT-stimulated cells particularly in lower doses at 0.06mg. *Mukta Bhasma* has significantly increased the intracellular reduction of NBT dye to formazan crystals by neutrophils confirming the intracellular killing property when compared to Normal Control & were near to Positive Control (PC). By NBT test, Mukta Bhasma confirms the immune-stimulating effect.

This stimulated immunity further confirms the phagocytic function of neutrophils by engulfing the candida cells & also confirms the candidacidal effect significantly which was near to PC value. MB 0.06mg showed significant (p<0.001) increase in the phagocytic activity, candidacidal activity and Neutrophil Locomotion and Chemotaxis test when compared to MB 1.0 mg. *Mukta Bhasma* significantly increased the movement of Neutrophils towards the foreign body which is most important step in phagocytosis process.

Probable mode of action of *Mukta bhasma*

Mukta (Pearl) is one of the precious stones. It is used in various diseases because of its properties like sweet (Madhura), cooling (sheeta virya) & unctuous (snigdha). By these qualities, it has strengthening (*balya*), rejuvenating (*aayushya*), immune-modulating (*rasayana*) & aphrodisiac (*vrishya*). Hence indicated in various conditions like immunosuppressed diseases, Respiratory conditions, demaciated conditions, Acid peptic disorders etc.

By the present study, we can attribute the concept of Acharya Charaka and Sushruta:

Kapha dosha (factor responsible for strengthening and lubricating the body) and Ojas (bioenergy that helps in sustaining of life) in normal balanced state stands for immunity. Here Mukta bhasma having above said qualities will increase Ojas and also increase Kapha dosha in balanced state which will serve the purpose of balya & rasayana(12,13). The outcome

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of whole present study can be attributed to the above said concept of balya, rasayana etc.

But in Ayurvedic parlence, Mukta is having wide spectrum of applications from Daivavyapashraya chikitsa (spiritual healing) to Yukti Vyapashraya chikitsa (rational therapy).

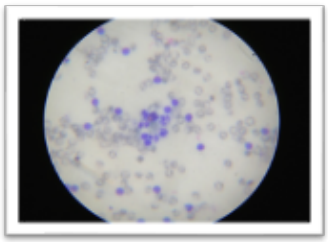
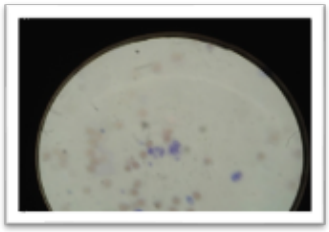
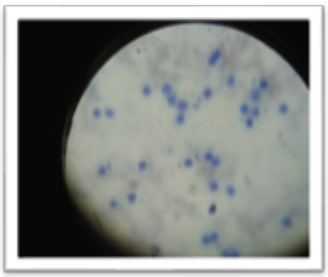
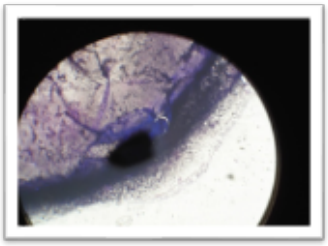
In modern view

Physiologically, the integrity & permeability of cell membrane is regulated mainly by Calcium which is abundantly present in Mukta bhasma (14). So, this fact may support in the prevention of cell damage when CCl4 was administered. it boosts innate immunity and helps regulate autoimmune responses Additional to Ca, Fe is also present in Mukta Bhasma which serves as a cofactor for the enzyme Catalase which detoxifies Hydrogen peroxide by dismutation to water & oxygen (15). By the present study, Mukta bhasm having antioxidant & free radical scavenging property mainly acts by inhibiting free radical mediated cell damage process.

Mukta Bhasma also showed immunomodulatory effect invitro. This helps in preventing the immune mediated hepatic damage especially in CCl4. It also helps in preventing the inflammatory process which occurs in the CCl4 toxicity.

Physiologically, the integrity & permeability of cell membrane is regulated mainly by Calcium which is abundantly present in Mukta bhasma(14). So, this fact may support in the prevention of cell damage when CCl_4 was administered. Mukta having high calcium content plays a crucial role in signal transduction pathways, contraction of various muscle cell types, the inhibition of cell proliferation, and the regulation of apoptosis.Also it boosts innate immunity and helps regulate autoimmune responses(15). Additional to Ca, Fe is also present in MB which serves as a cofactor for the enzyme Catalase which detoxifies Hydrogen peroxide by dismutation to water & oxygen(16). By the present study, Mukta Bhasma having antioxidant & free radical scavenging property mainly acts by inhibiting free radical mediated cell damage process.

Figure 8: Immunomodulatory study photos

NBT stimulated cells	NBT unstimulated cells	Phagocytosis	Chemotaxis movement
			

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