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COMPARISON OF THE TOXICOLOGICAL EFFECT OF LEAD-BASED HERBO-MINERAL PREPARATIONS AND THEIR CORRESPONDING METAL NANO-PARTICLE ON ENZYMATIC ACTIVITY AND GROWTH OF BAKER'S YEAST

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ABSTRACT: Metallic toxicity associated with Ayurvedic formulations like herbomineral preparation (Bhasma), especially because of heavy metals such as lead, has always been a matter of debate in the scientific community. Instead of that, ancient Ayurvedic literature reveals that sodhana process used for the preparation of such formulations, either decreases or eliminate the toxic effect of such heavy metals up to a negligible extent. But, the method for their standardization should be well defined. In present work, marketed lead preparation (Bhasma), In-house prepared lead preparation, and their corresponding micro/nano-particle have been evaluated for their physiochemical properties and toxicological impact. For the toxicity studies, instead of animals, two different methods like enzymatic inhibition and change in the growth pattern of yeast cells were explored to find out and establish the toxicity of such preparations. The results of the study reveal that sodhana process significantly effects to the physicochemical character of the metallic preparation and presence of heavy metals like lead effects to the activity of enzymes and growth pattern of the yeast and can successfully be used for the *in-vitro* toxicity studies.

INTRODUCTION: Toxicity associated with various metals has raised doubt over the number of herbo-mineral preparations which are being since the ancient time in Ayurveda and started a never-ending debate on the safety and efficacy of bhasma [1, 2]. Lead is one of the most notorious elements known for its toxicity, and there are several cases have been reported regarding the same [3]. But, it has also been mentioned that there are several herbo-mineral formulations such as lead bhasma which do not have ill impact [4, 5].

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Literature survey reveals that almost every Ayurvedic preparation contains at least one metal while the herbo-mineral preparations used in Ayurveda are proven to have several elements in different combinations [6]. Several other traditional medicines are also there, other than Ayurvedic medicine belonging to Asian, Middle Eastern and Hispanic cultures that contain several heavy metals like lead, mercury, *etc*. [7, 8] A study reveals that prolonged use of the Ayurvedic formulation containing lead causes abdominal pain, nausea and bilious vomiting [9, 10].

There is one lead-based Chinese traditional medicine (Ba-pao-neu-hwang-san) has also been reported, which is thought to be safe even in pediatric patients too [11]. In different formulations, lead is either present in divalent state or tetravalent state, while the divalent state is regarded as more

stable and less toxic. Literature reveals that herbo-mineral preparation containing lead *i.e*. lead bhasma mainly contains galena (PbS) owing to the more stability and less toxicity [12, 13].

Lead imparts its toxicity by inducing the oxidation process [14]. Lead increases the oxidized glutathione in spite of reduced glutathione leading to the increased oxidative stress, finally leading to the lead-based toxicity [12, 15, 16]. Apart from the above, lead also inhibits to the activity of superoxide dismutase and catalase too, which is another very important reason behind the lead toxicity. Lead also can replace various bivalent ions like zinc, calcium, magnesium, *etc*. which works as co-factor for various enzymes which play an important role in homeostasis [17]. Lead also tends to replace calcium ions and get easily permeated to the brain leading to the neurotoxicity. In some cases, symptoms related to encephalopathy like anxiety, delirium, muscle weakness, tremor, convulsion, insomnia, *etc*. has been observed due to high dose exposure of lead [18]. A study shows that exposure towards the lead in the fetus has resulted in decreased birth weight, deformity in development of skeletal structure and neuro-toxic effects too [19-21].

Lead activates or induces the expression of new genes by activating protein kinase C in intact cells leading to the impairment in the learning process [22, 23]. The possibility of lead nephropathy followed by hypertension due to tubule-interstitial nephritis is another major challenge during the treatment of patients with lead-based herbo-mineral formulation [24]. There are various enzymes such as δ-Aminolevulinic acid dehydratase (ALAD), Aminolevulinic acid synthetase (ALAS) and Ferrochelatase, which are mainly responsible the heme biosynthesis are largely affected by the lead poisoning [25, 26]. Decreased libido, negative impact on spermatogenesis, dysfunctioning of prostrate, abrupt changes in chromosomal pattern, *etc*. are some more major toxic effects imposed by the consumption of lead-based formulation for longer duration in higher dosage [4, 23].

There are several cases of lead toxicity has been reported due to the uses of traditional herbo-mineral preparation used for the longer time [27, 28]. Sodhana process adopted for the preparation of such formulation plays a detrimental role with respect to their safety, efficacy, and toxicity [29, 30]. It has been noticed that improper sodhana of such formulation is one of the major reason behind the toxicity of heavy metals [7, 31]. Presence of lead and other metals has also been reported in various marketed herbo-mineral preparation [10, 32]. As far as the therapeutic use of the lead-based herbomineral preparation like lead bhasma is concerned, it is being used since a very long time and find effective and non-toxic in various studies [33]. But based on above discussions toxicity related to such preparation having lead cannot be ignored and should be studied exhaustively. Several animal models have been reported to check out the toxicity of such materials [34-37] but still, we are lacking with enough sensitive methods for checking the toxicity without using the animals. In present work, an attempt has been made to develop the methods for finding the toxicity of such preparation without animal models using baker's yeast [38, 39], different digestive enzymes and change in the protein behavior.

MATERIAL AND METHOD: Naga (lead) bahsma (Marketed) was procured from the local Ayurvedic store (Batch No.-P160200265), and one sample of bhasma (prepared Nag bhasma) has been prepared with the help local Ayurvedic practitioner (RRS-5/172-182). While blend of lead oxide micro/nano-particles were prepared in the institute laboratory. Bakers yeast, Pepsin, and Diastase were procured from a local vendor.

Characterization of Herbo-Mineral Preparation and Corresponding Nano-Particle:

XRD Studies: Powder XRD of herbo-mineral preparation and corresponding bhasma was performed at IISER, Bhopal using PANalytical Empyrean XRD X-ray diffractometer with the parameters using CuKα radiation, l = 1.5406 Å over the range 5.203º 80.148°.

IR Spectral Analysis: The IR analysis was performed with spectra measured over the frequency range 500-4000 cm^{-1} using Bruker's FT-IR at RGPV, Bhopal.

Particle Size Analysis: The particle size of both the preparation was found out using Zetacizer (Horiba). Samples were suspended in the 0.25% Carboxymethylcellulose solution to avoid the

settling of the particle during the study. SEM studies were also performed to find out the size as well as surface behavior of the particles.

Study of Toxicological Impact of Bhasma using the Standard Procedure of Pepsin Activity (I.P): According to IP 1996, the activity of pepsin can be assayed by finding its ability to digest the egg protein. For continuing with the study, 0.05 g of pepsin was accurately weighed and triturated with 200 mg of sodium chloride with slow addition of acidified water and make up the volume up to 200 ml with continuous shaking up to 15 min. Further, 3 g of coagulated egg albumin already passed through sieve no. 44 was taken and mixed thoroughly with 10 ml of the acidified water, ensuring that the particles of egg albumin are completely disintegrated. Ten ml of acidified water was further added to it and kept in a water bath at 51 °C temperature for 15 min. After that 4.0 ml of an already prepared solution of pepsin was added and kept the entire material on 51 °C temperature again for the digestion until 4 h with intermittent shaking at the intervals of 15 min. After completion of digestion, the complete suspension was centrifuged, and the supernatant was decanted off. The remaining material was washed into a 10-ml graduated cylinder and allowed to stand for 30 min [40].

Study of Toxicological Impact of Bhasma using the Standard Procedure of α-amylase (diastase) Activity (I.P): One gm of fungal alpha-amylase (diastase) was weighed accurately and triturated with 2 ml of acetate buffer (pH 5) with the further addition of sufficient acetate buffer pH 5 to produce 10 ml. Further, this solution was diluted up to 100 ml with acetate buffer pH 5 and filtered. Further, 5 ml of a starch solution prepared as per the monograph and added into the test-tube without touching the sides of the test-tube and placed in a water-bath maintaining the temperature at 40° ± 0.1 °C. 1 ml of already prepared diastase solution was added to the starch solution and again kept in the water bath to maintain the temperature. After heating the above to 60 min, it has been cooled rapidly in cold water and added 0.05 ml of iodine solution and mixed well. For finding the inhibitory effect of the herbo-mineral preparation on the diastase activity, standard diastase solution was treated with bhasma for 30 min and then the capability of change in starch digestion was recorded [41].

Toxicological Study using Baker's Yeast: Growth of yeast was obtained using Yeast extract, peptone, and dextrose media. For the cup plate method solid agar plate using YPD and for suspension culture, liquid YPD media was used. All the ingredients were dissolved in 1 L of distilled water and sterilized by autoclaving at 121 °C and 15 mps for 20 min. Combination of the ingredient 1, 2 & 3 of **Table 1** was taken to prepare the liquid YPD media while solid agar plate of YPD media was prepared using all the four ingredients [42].

TABLE 1: COMPOSITION OF YEAST EXTRACT, PEPTONE, DEXTROSE (YPD) MEDIA USED FOR THE STUDY (LIQUID OR SOLID)

S. no.	Reagent	Amount
1	Yeast extract	10 g
2	Peptone	20 g
3	Dextrose	20 g
4	Agar (for plates only)	20 g

Cup-Plate Method: Solid agar YPD (yeast extract, peptone, and dextrose) media was prepared and sterilized and after that baker's yeast was added during the cooling and solidification in aseptic condition. Three different plates were prepared and the cup was made for the addition of the material to be checked for the toxic study [43, 44]. Three different plates are prepared-

Set of Plate 1: Cup was seeded with sterilized normal saline.

Set of Plate 2 & 3: Cup was seeded with a sterilized suspension of bhasm (marketed and prepared) in normal saline.

Set of Plate 4: Cup was seeded with a sterilized suspension of microparticles in normal saline.

All the plates were incubated at 37 ± 0.5 °C, and the zone of inhibition was observed.

By Suspension Culture: Impact of bhasma and corresponding nano-particle was studied on the growth and morphology of the baker's yeast. Yeast suspension was prepared using YPD (yeast extract, peptone, dextrose) media [45] aseptically and divided into three different sets.

Set 1: YPD media with yeast.

Set 2 & 3: YPD media with yeast treated with bhasma (Marketed and prepared).

Set 4: YPD media with yeast treated with nano-particle.

Ten vials of each set were taken and incubated at 37 ± 0.5 °C in shaker incubator, and optical density (OD_{590}) of the yeast suspension of each set was measured at 590 nm using the corresponding blank. The graph between the time and OD_{590} was plotted to prepare the growth graph. Change in the growth curve pattern and change in the microscopic view of the yeast cell was observed [46].

RESULTS AND DISCUSSION: The FTIR spectrum for prepared lead bhasma as mentioned in **Fig. 1A** showing the peak at 3547.09 cm^{-1} corresponds to free hydroxyl group, which may be due to the presence of moisture. A broad peak at 1417 cm^{-1} shows the presence of sulfate group, a peak at 1045 cm^{-1} corresponds to the sulfoxide while a peak at 530.42 cm^{-1} shows the presence of disulfide bond and a peak at 686.66 cm^{-1} shows the presence of metal carbonate, probably lead carbonate. A characteristic band at 840 cm^{-1} represents the presence of lead sulfide. Several bands in the range of 400 to 650 cm^{-1} (424.34 cm^{-1}, 459.06 cm^{-1}, and 640.37 cm^{-1}) show the presence of oxides of metals, which is probably lead oxide in this case.

While the FTIR spectrum of marketed lead bhasma as mentioned in **Fig. 1B** has shown several peaks in the range of 3664.75-3784.34 cm^{-1} which may be attributed to the presence of free O-H group [47]. The present sample has shown the prominent peaks at 596 cm^{-1} and 1055.06 cm^{-1} and in between 950-100 cm^{-1} and 1165-1750 cm^{-1} showing to the presence of anglesite [48].

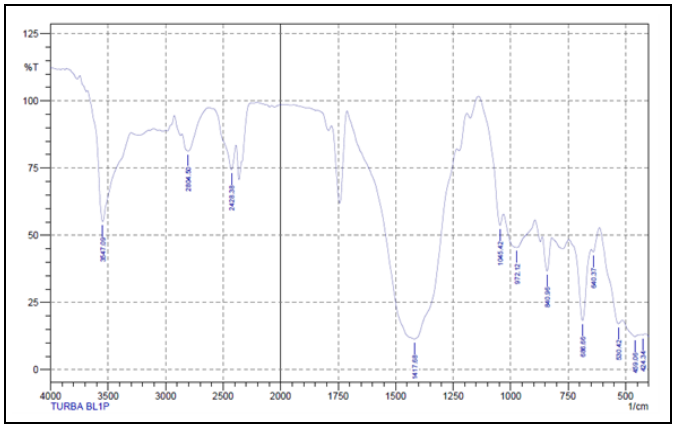

FIG. 1A: FTIR SPECTRA OF PREPARED LEAD BHASMA

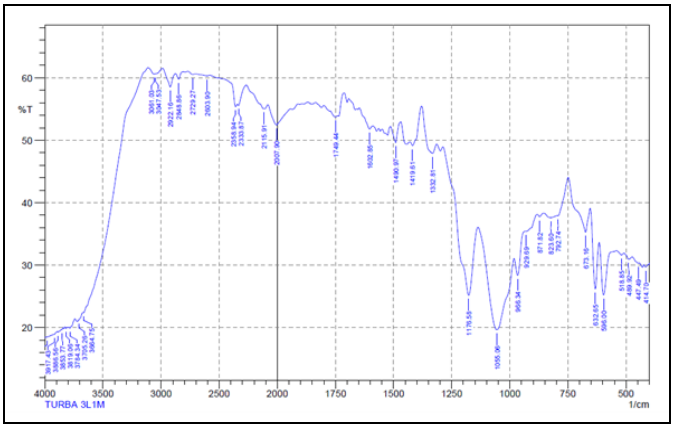

FIG. 1B: FTIR SPECTRA OF MARKETED LEAD BHASMA

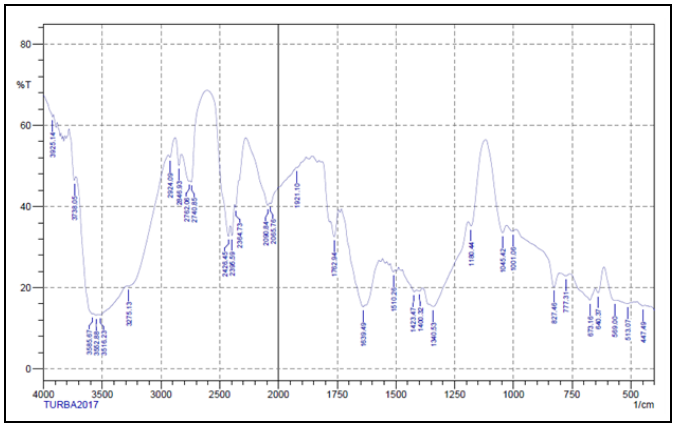

FIG. 1C: FTIR SPECTRA OF PREPARED LEAD MICRO-PARTICLES

IR spectral Bands for $PbSO_3$ were also observed at 871.82 cm^{-1}, 929.69 cm^{-1} and 966.34 cm^{-1} [49]. The peaks at 823.60 cm^{-1} and 871.82 cm^{-1} indicates the bands for Pb-S [50]. The peak at 632.65 cm^{-1} is due to the S-S bond and the peak at, and peak below 500 cm^{-1} shows the presence of metal oxides, which is

probably lead oxide in this case. While peak at 1332.81 cm^{-1} corresponds to the presence of sulfons. Presence of a weak peak area in the range of 3700-3750 cm^{-1} with a characteristic peak at 2333.87 cm^{-1} (NH_3) and 2358.94 cm^{-1} (double band) shows the presence of nitrogen-based materials in sample [51].

IR spectra of lead micro-particulates, as shown in **Fig. 1C** have a broad peak ranging 3516.23-3585.67 cm^{-1} may be due to the presence of OH groups. It can also be confirmed by the presence of bands near 1400 cm^{-1} showing bending –OH (maybe because of absorbed water). An intense peak at 673.16 cm^{-1} may be due to the presence of Pb-O-Pb like system. Presence of PbO may be confirmed with the presence of a peak at 447 cm^{-1}. [52]

In the X-diffraction study, peak identification and matching were performed by Match (version 3.7.0.124) software and the major X-ray diffraction peak in case of in-house prepared bhasma, as shown in **Fig. 2A** was observed at 2θ = 20.75, 26.31, 26.47, 27.17, 28.60, 30.74, 31.79, 33.53, 34.08, 35.98, 43.69, 47.46, 52.03 and 52.15 corresponding to lead (IV) oxide (Pb_3O_4), Ammonium Lead Triiodide dihydrate ($NH_4PbI_3(H_2O)_2$), Silica zeolite ($Si_{32}O_{64}$), and Lead sulfide (PbS).

Sharp and high-intensity XRD peaks indicate the presence of these materials in crystalline form with a different structure like tetragonal, orthorhombic, and monoclinic, as shown in the figure. While in case of marketed bhasma **Fig. 2B** major X-ray diffraction peak was observed at 2θ = 14.52, 25.96, 26.71, 43.24, 47.76, 50.69, 62.52, 67.23 and 71.2 representing to Galena (PbS), Anglesite ($PbSO_4$), fassinaite ($Pb_{22}+(S_2O_3)(CO_3)$), Periclase (MgO), Sussanite ($Pb_4SO_4(CO_3)_2(OH)_2$) and iron as shown in the **Fig. 2B**.

Sharp and high-intensity XRD peaks indicate the presence of crystalline with cubic, orthorhombic, and trigonal structure. XRD pattern of prepared microparticles of lead oxide **Fig. 2C** has shown the peak at 2 θ=16.68, 17.58, 20.30, 27.62, 34.12, 41.74, 44.46 and 53.14 repressing to the monobasic lead sulfate ($PbO \cdot PbSO_4$), di-basic lead sulfate ($2PbO \cdot PbSO_4$), NaPb, lead (Pb) and Susannite ($Pb_4SO_4(CO_3)_2(OH)_2$).

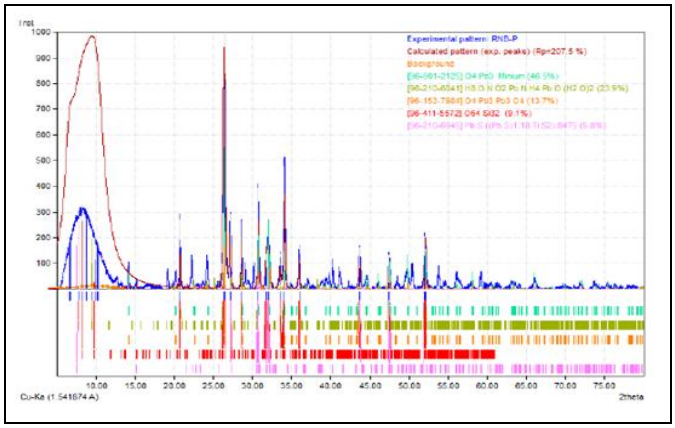

FIG. 2A: XRD SPECTRA OF IN-HOUSE PREPARED LEAD BHASMA

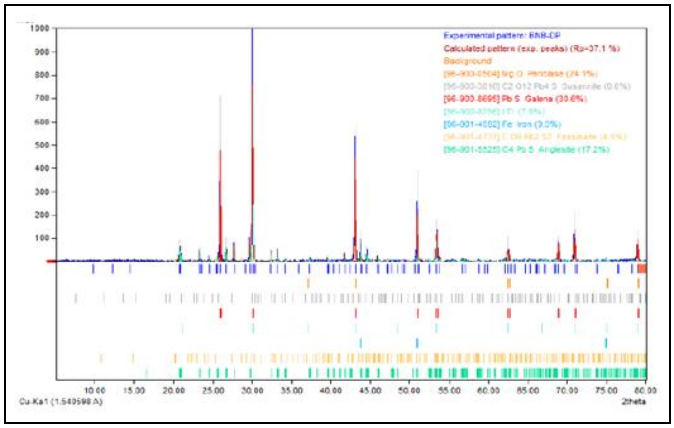

FIG. 2B: XRD SPECTRA OF MARKETED LEAD BHASMA

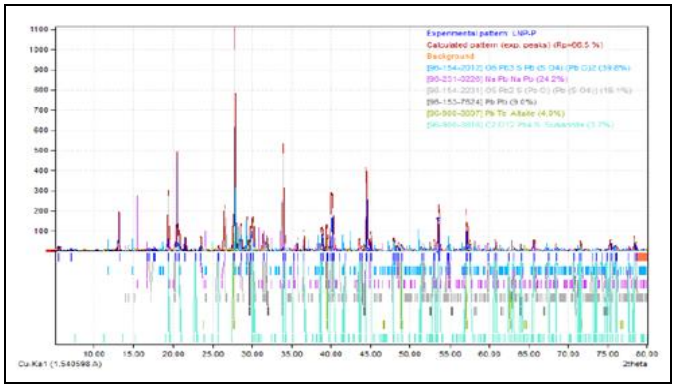

FIG. 2C: XRD SPECTRA OF PREPARED MICROPARTICLES

The sharp peaks represent the crystalline form of these materials, especially in orthorhombic, tetragonal, trigonal, monoclinic, and hexagonal. It was observed that in comparison to the bhasma, nano-particulate material has mixed form, *i.e.* trigonal, orthorhombic, cubic and tetragonal structure. At the same time Bhasma preparation lack with the free lead particles rather than the nanoparticles.

Morphological study of herbomineral preparation and prepared nano-particles using scanning electron microscopy revealed that the marketed bhasma formulation has almost round or oval de-aggregated particle structure with smooth surfaces as shown in **Fig. 3A**.

While the prepared lead bhasma formulation shows some smaller aggregates with flakes like structure at the surface of larger particles. As shown in **Fig. 3B**, needle, and oblong crystal have also been found during the study.

SEM study of micro-particulates of lead, as shown in **Fig. 3C** has the presence of aggregate like structure with the presence of flakes like the system at the surface of larger particles.

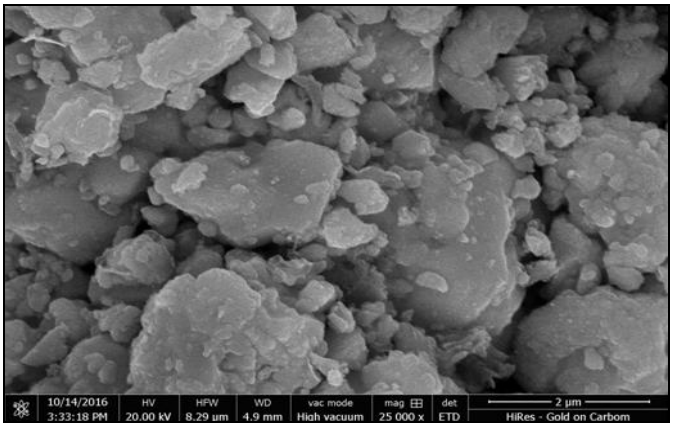

FIG. 3A: SEM PHOTOGRAPH OF MARKETED LEAD BHASMA

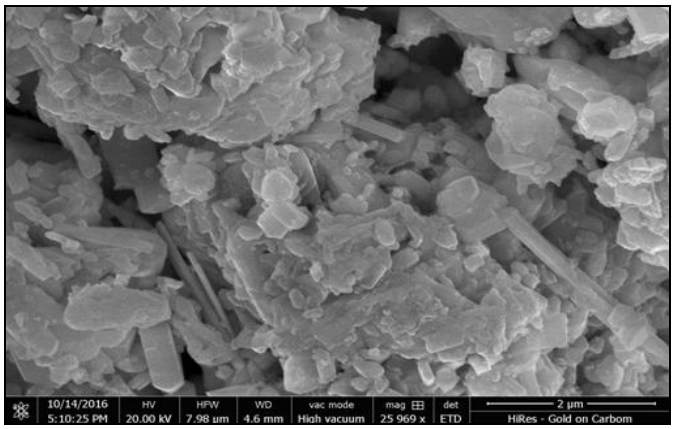

FIG. 3B: SEM PHOTOGRAPH OF PREPARED LEAD BHASMA

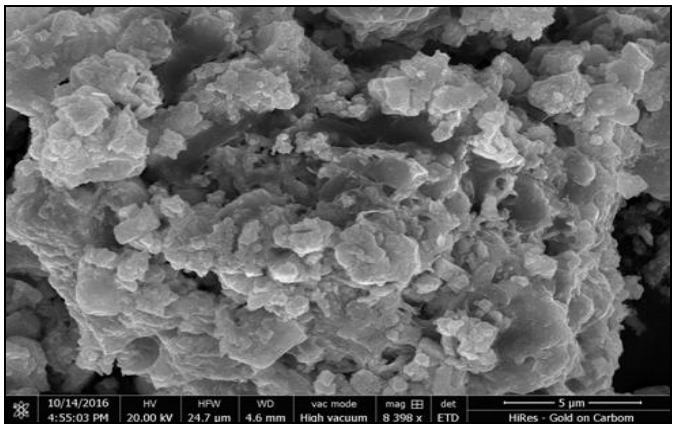

FIG. 3C: SEM PHOTOGRAPH OF PREPARED MICRO-PARTICLES OF LEAD

To collect the nanosize particles in the formulations which are likely to be absorbed in the intestine, formulations were filtered through a 0.45 µm filter. When these formulations were filtered through a 0.45 µm filter, the average size of majority of particles which was retained on the filter paper was found to be less than 7.2 µm in marketed bhasma formulation while the prepared bhasma contains average particle size less than 11.4 µm with a yield of 33.6 and 36.4% respectively. Prepared micro-particles have shown the average size of 7.6 µm with a percentage yield of 33.8%. When the average size of filtrate of each system was studied, it was found to be 105 nm, 147 nm, and 152 nm respectively for Marketed lead bhasma, prepared lead bhasma and prepared micro-particulates respectively.

Studies reveal that inhibition of enzymatic activity by various heavy metals is one of the important

reason for heavy metal toxicity. To find out the impact of lead-based herbomineral preparations, their inhibitory effect was studied on two different enzymes (pepsin & diastase) by their standard assay protocol (IP 1996).

As mentioned in **Table 2**, It was observed that in comparison to marketed bhasma, nano-material, and prepared bhasma formulation has shown an inhibitory effect on the pepsin activity (I.P 1996).

TABLE 2: PEPSIN ACTIVITY ON DIGESTION OF PROTEIN IN DIFFERENT CONDITION

S. no.	Metals + pepsin	The total volume of test material containing protein	Precipitated volume after centrifugation
1	Standard	10 ml	0.5 ± 0.2 ml
2	Marketed Naga bhasma	10 ml	1.4 ± 0.2 ml
3	Prepared Naga bhasma	10 ml	2.0 ± 0.1 ml
4	Lead Microparticle	10 ml	1.7 ± 0.2 ml

This inhibitory effect may be due to the presence of free lead ions, which are supposed to block the activity of pepsin **Fig. 4A**.

FIG. 4A: IMPACT OF DIFFERENT PREPARATION ON THE PEPSIN ACTIVITY ON EGG PROTEIN (A) PEPSIN TREATED WITH LEAD MICROPARTICLES (B) PEPSIN TREATED WITH MARKETED LEAD BHASMA (C) PEPSIN TREATED WITH MARKETED LEAD (D) BHASMA UNTREATED PEPSIN

As XRD and IR studies reveal and support the presence of lead sulfide in the marketed lead formulation, which is thought to be the safest form of the lead while prepared bhasma has a lead oxide (PbO) and lead sulfate ($PbSO_4$) which may be responsible for the generation of free lead ions. During diastase activity on starch paste, it was observed that pre-treatment of diastase with marketed bhasma shows an increase in catalytic property in the digestion of starch which has no clear mechanism, but it may be attributed to the properties of the herbs which are being used during the preparation of bhasma. But at the same time, lead micro-particles have also shown the incremental impact on the activity of diastase due to unknown reasons.

In contrast to it, prepared bhasma formulation has a comparative inhibitory effect on diastase as it has taken more time to digest starch in comparison to the untreated diastase but as such, there is no marked change in the activity of diastase by the marketed bhasma formulation too **Fig. 4B**.

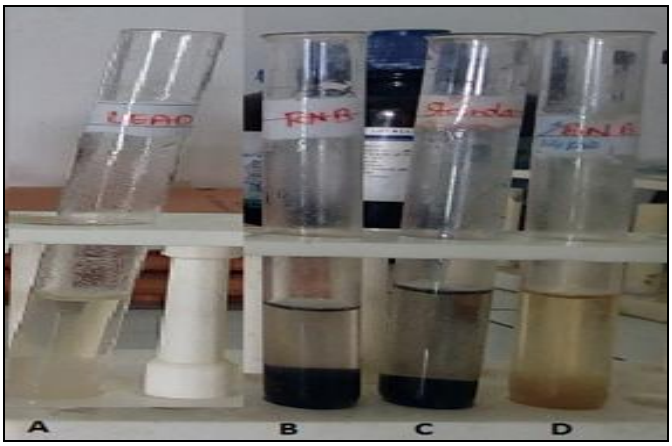

FIG. 4B: IMPACT OF DIFFERENT PREPARATION ON THE DIASTASE ACTIVITY ON STARCH A) DIASTASE TREATED WITH LEAD MICROPARTICLES B) DIASTASE TREATED WITH PREPARED LEAD BHASMA C) UNTREATED DIASTASE D) DIASTASE TREATED WITH MARKETED LEAD BHASMA

Microbiological assay, using cup-plate method has shown that prepared bhasma and lead nano-particulate formulation both have shown inhibitory action in comparison to the control plate loaded with normal saline, while marketed bhasma formulation has no major effect on the growth of yeast cells as shown in **Fig. 5**.

Control plate seeded with 0.9% NaCl showed tremendous growth within 48 h and forms a smear like structure on the entire plate including the cup. While the two different plates seeded with 10 mg of prepared bhasma and 10 mg of nano-particle shows growth inhibition demonstrated by the clear black patch (may be due to the internalization of lead nano-particles or due to change in pH of the microenvironment) as shown in **Fig. 5**.

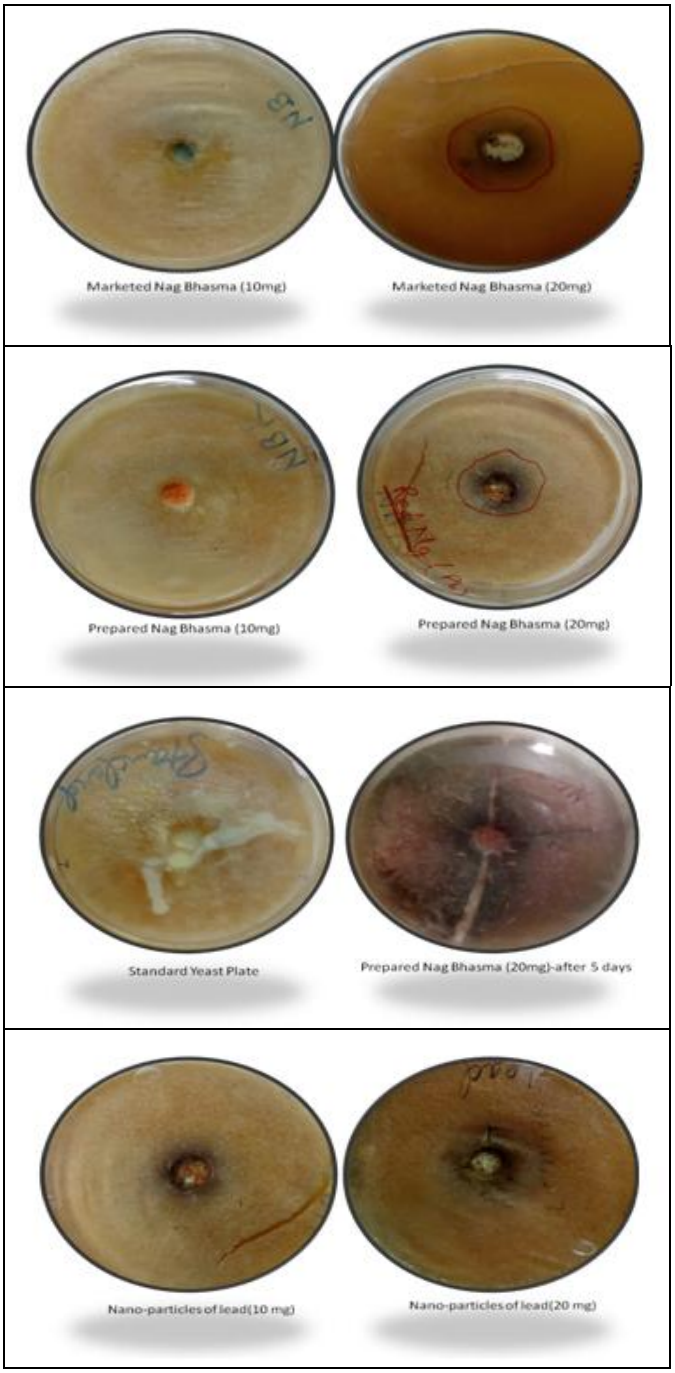

FIG. 5: YPD AGAR PLATE SEEDED WITH YEAST CELLS IN DIFFERENT CONDITIONS

At the same time, plate seeded with marketed bhasma has shown no major change in the growth of yeast cells. At a higher amount (20 mg), micro-particles shows a similar pattern and extent of inhibition as compared to 10 mg of particulate material while the increased concentration of prepared bhasma has shown almost negligible growth including the complete plate which shows high inhibition effect of the same. In contrast to that, increased concentration of marketed lead bhasma has no major change in comparison to the 10 mg of its concentration. A smaller change in the growth of yeast cells may be due to the development of a micro-osmotic area due to the presence of several other elements like Pb, Na, Mg, Si, *etc*. But still, in case of lead particulate matters and marketed lead bhasma, no clear conclusion may not be drawn regarding the toxicity.

So, in the further study it was planned to find out the cumulative growth of the yeast cell for a certain period in YPD media as the micro-osmotic pressure generation due to the several other inorganic materials will be distributed to the larger area and almost it will be negligible as a comparison to the cup-plate method.

Being eukaryotic in nature, bigger in size and non-pathogenic in nature, yeast cells have been chosen to find out the toxicological impact of the above materials. Due to their surface behavior, dividing plane, and budding process, it also provides the information about the metabolic process of the cells and any disturbance or change during this process indicates the inhibition of normal cell process indicating the toxicity. In the present study, at regular time interval Growth of the yeast cell was measured by using increment in the optical density.

The growth curve was plotted using time and optical density of the cell. It has been observed that in all cases, the growth rate of the yeast has been hindered in comparison to the standard yeast suspension. In **Fig. 6**, it can easily be observed that the yeast growth in the presence of prepared bhasma and prepared microparticles has attained the saturation phase little bit early. While in the case of marketed bhasma formulation, yeast has shown better growth pattern similar to the standard yeast cells.

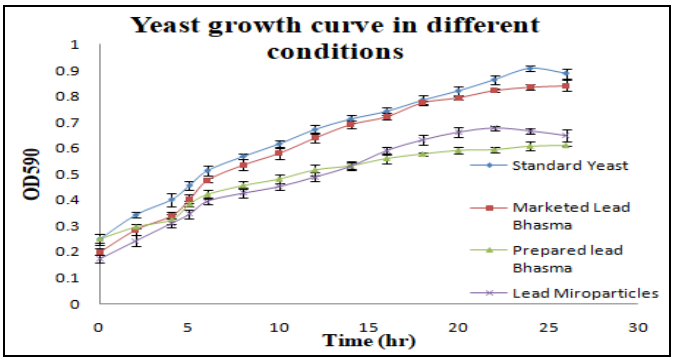

FIG. 6: YEAST GROWTH CURVE IN DIFFERENT CONDITIONS

From **Fig. 6**, it can be observed that the growth of yeast cell has been hindered by the prepared bhasma and prepared microparticles supporting to the previous studies but still as the growth has been observed it cannot be said that these materials are toxic in the particular concentration.

Morphological study of the yeast cell in different condition as mentioned in **Fig. 7** has shown that there is no major change in the structure of yeast cells that occur during their incubation in standard condition, as mentioned in **Fig. 7A**, while the presence of prepared lead bhasma has significantly affected the yeast cell morphology leading to the damage of cells **Fig. 7B**. This damaging of the cell may be attributed to the presence of lead sulfate ($PbSO_4$) causing a change in the osmotic environment of the cell after engulfment by the cells. Internalization of the bhasma particles in case of marketed preparation may also be observed in **Fig. 7C**. It has been noticed that normal yeast cells have a more regular and uniform structure than the yeast cells treated with different formulations.

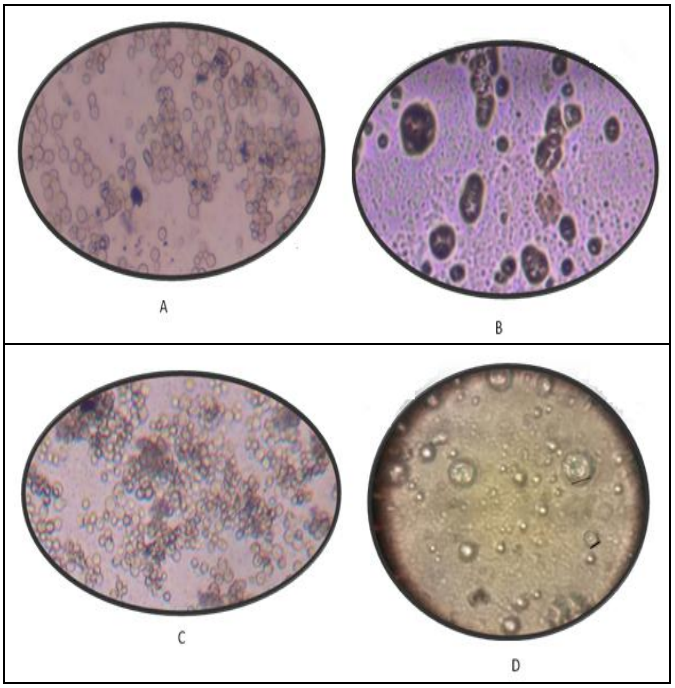

FIG. 7: MORPHOLOGICAL CONDITION OF YEAST CELLS IN DIFFERENT SITUATIONS A) STANDARD YEAST CELLS B) YEAST CELLS INCUBATED WITH PREPARED LEAD BHASMA C) YEAST CELLS INCUBATED WITH LEAD MICROPARTICLES D) YEAST CELLS INCUBATED WITH MARKETED LEAD BHASMA

During the study, it was observed that the yeast cells uptake herbo-mineral particulates with a change in their size, which is clearly visible in **Fig. 7D**. Similarly, it can also be seen in **Fig. 7C** that the metallic micro-particle (Prepared in-house) has also been taken up by the cell leading to the aggregation of the yeast cell to form a bunch or it may be due to the agglomeration of yeast cell around the particle. Some changes in the cell structure were also observed.

CONCLUSION: In the recent decade, the use of Ayurvedic perpetration for the treatment of ailments has been increased tremendously, and getting the acceptability as an important system in the healthcare industry. Presence of lead and other heavy metals in the different Ayurvedic formulation has always been a matter of debate due to their toxic nature. But at the same time, some lead containing or lead-based formulation like nag bhasma is also there which are being used in the Ayurvedic system.

Literature in the Ayurvedic system also reveals the fact that after proper sodhana process can eliminate the toxic or ill effect of such materials. It has also been observed that the physiochemical nature, as well as the morphological characters of the metal particles, get changed. But, in the present time, it is utmost needed to have some specific methods used for accessing toxicity, if any. In present work, we have tried to prove the impact of sodhana process on the physiochemical characteristic of such metallic preparation.

We have also tried to utilize the enzymatic assay method, microbiological assay, and yeast growth pattern to establish the toxicity in spite of animal studies. As the yeast cells are eukaryotic, significantly have a larger size, and show a significant change in the morphology due to the presence of toxic material. So, change in the morphology of yeast cell due to the presence of different material can also be used to access the impact of sodhana process. The results have shown that above mentioned three methods can successfully be used to find out the toxic impact of the heavy metals or related compounds which are likely to be present in various Ayurvedic preparations.

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