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A Comparative Study to Evaluate the Physico-Chemical Characteristization and Blood Compatibility Studies of Tāmra Bhasma (Incinerated Copper Particle) With and Without Amritīkarana

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KEYWORDS

Tamra bhasma, Amritikarana, Zeta potential, DLS, ICP-OES

ABSTRACT:

Introduction: Extensive utility of Tāmra bhasma has been mentioned in Rasa classics, Tāmra is supposed to be a poison or more than a poison if used in ashudha (impure) form. According to Ayurveda, Tāmra bhasma is told to be used only after a special procedure called amrİtīkarana. AmrİtīkaranaAmrİtīkarana is a special procedure carried out to eliminate the remaining doshas of any dhatu bhasma and increases therapeutic effect of bhasma. AmrİtīkaranaAmrİtīkarana removes the ashta doshas (eight ill effects) of Tāmra.

Objectives:

- To analyse the physico-chemical parameters of Tāmra bhasma prepared with and without AmrİtīkaranaAmrİtīkarana.
- To experimentally evaluate Cytotoxicity of Tāmra bhasma with and without amritīkarana.
- To experimentally evaluate blood compatibility of Tāmra bhasma with and without amritīkarana.

Results and discussion: Both samples have particles ranging from nanometer to micrometer level. DLS results shows that the particle size of Tāmra bhasma was reduced after amrİtīkarana. The results suggests that the sample is in polydisperse state. Zeta potential of TB and TBA shows that TBA sample is more stable than TB. Elemental analysis by ICP-OES results shows that mercury content in both samples were found to be within the limit. Arsenic content was reduced in TBA sample comparing to TB. Chemically TB is identified as Cu12As4A13 in Cubic crystalline form and TBA is identified as Cu12As4A13 in Cubic crystalline form and Cu9S5 in Rhombohedral crystalline form. Both samples are non- cytotoxic. Maximum and minimum cell activity was seen at concentration 0.0625 mg/ml and 1 mg/ml for both samples. Both samples showed cytocompatibility at lower concentrations. TB cells shows slightly increase viability at larger concentration. The hemolysis induced by TB and TBA was 0.04% which was well within the acceptable limits of 1%. Based on hemolysis value we can assume that plasma hemoglobin level of samples are also normal. Change in values of platelet count was shown in both samples. Comparing to TB, TBA shows more variation. Measurement of uncertainty acceptable value is 5% but here, 10% change is shown for sample TBA. This shows that TBA encourages platelet adhesion so, this can be used in bleeding disorders. The control plasma and the plasma containing the TB sample shows almost equal amounts of fibrinogen and partial thromboplastin time. But TBA sample shows increased value in results comparing to the control. Despite the observed difference, no delay in coagulation time was observed in plasma samples. Activation of platelets initiates the deformation of the cells with pseudopod formation and ends with blood coagulation or thrombus formation. In the present study platelets seem to be not activating indicates platelet compatibility of both samples.

Conclusions: For providing necessary scientific validation for structure and composition of Tāmra bhasma prepared with and without amritīkarana, physico-chemical parameters and advanced instrumental analysis

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were done. From this study it is evident that amritīkarana helps in making Tāmra bhasma safer based on the physio-chemical parameters. As per methodology, Tāmra bhasma prepared without amritīkarana showed better results in blood compatibility.

1. Introduction

From Vedic period onwards, metals were used in several disease conditions, but their use increased after progress of *Rasaśāṣtṛa*, which is an integral part of *Ayurveda*. *Bhasma* (ash) of metals or minerals are one of the appropriate pharmaceutical forms in *Rasaśāṣtṛa*. *Tāmra bhasma* is one among them. The metallic or mineral preparation formed after treating with herbal juices or decoction and then exposed to quantum of heat (puta) is called as bhasma.[1] *Tāmra bhasma* is derived from metallic copper and is used for the treatment of abdominal pains, heart problems, different ailments of liver and spleen, colitis, anaemia, tumors, dropsy, eye troubles, loss of appetite tuberculosis etc.[2] Even though extensive utility of *Tāmra bhasma* has been mentioned in *Rasa* classics, *Tāmra* is supposed to be a poison or more than a poison if used in *ashudha* (impure) form. According to Ayurveda, Tāmra bhasma is told to be used only after a special procedure called amrİtīkarana. AmrİtīkaranaAmrİtīkarana is a special procedure carried out to eliminate the remaining doshas of any dhatu bhasma and increases therapeutic effect of bhasma. AmrİtīkaranaAmrİtīkarana removes the ashta doshas (eight ill effects) of Tāmra.[3]

2. Materials and Methods

2.1 Pharmaceutical preparation

Two samples of Tāmra bhasma with and without amrİtīkarana was prepared in Department lab of *Rasaśāṣtṛa* and bhaishajya kalpana, Amrita School of Ayurveda and designated as TB and TBA respectively. The pharmaceutical processing of Tāmra bhasma includes samanya śodhana (general purification methods), viśeṣa śodhana (specific purification), māraṇa (incineration) and amrİtīkarana. In the process of māraṇa and amrİtīkarana an electric muffle furnace (EMF) was used. Tāmra patra (Copper sheet) was heated till red hot and quenched subsequently into the tila taila (sesame oil), takra (curd), gomūtra (cow's urine), kāñcikā (fermentative preparation) and kulattha kvātha (decoction prepared with horse gram), 7 times each. This process of heating red hot and quenching in liquid media is termed as nirvāpa. Liquid medium was changed after 3rd nirvāpa and then washed in hot water. Viśeṣa śodhana was done by boiling Tāmra patra in gomutra (cow's urine) for 3 hours. Then Tāmra was washed, dried, and stored.[4]

For māraṇa (incineration) procedure Śuddha Pārada (purified Mercury) and Śuddha Gandhaka (purified Sulphur) are taken in a khalva yantra and triturated thoroughly to obtain appropriate kajjalī. Śodhana was done as per classical reference. To this Kajjalī, Śuddha Haratala (purified Orpiment) and Śuddha Manahshila (purified Realgar) are added and thoroughly triturated to obtain homogenous mixture. To a clean śarāva (earthen saucer), Kajjalī was added, over that Śuddha Tāmra patra was placed and above that again Kajjalī was added. Like this, layers of Kajjalī and Tāmra patra are put and at the end, all the remaining Kajjalī was heaped up to cover all the Tāmra patra. Then the śarāva was closed with another śarāva and sandhibandhana done (junction was sealed by multifolded clothes which were smeared with *multani mitti*).[4] After drying the Śarāva saṃpuṭa done was carefully kept in muffle furnace and heated for continuous 12 hours. The pattern of heat given for incineration is shown in table 1.

For amrİtīkarana one part of Tāmra bhasma and half part of Śuddha Gandhaka are taken in a clean khalva yantra (Mortar – pestle) and triturated homogenously. The mixture was added with required quantity of Paňcāmrİta (mixture prepared with cow's milk, curd, ghee, sugar and honey) and triturated thoroughly to prepare cakrikās (Pelletization) of even shape and size. The cakrikās are dried under sun, enclosed in śarāva saṃpuṭa and subjected for bhasmikarana in muffle furnace. The entire process was repeated thrice.[5] The temperature pattern for amrİtīkarana is shown in table 1.

. Table 1 showing temperature pattern and duration for preparation of TB and TBA

Temperature	Time taken to reach that temp.	Duration	
		TB	TBA
250°C	13 min	4 hours	2 hours
450°C	12 min	4 hours	2 hours
630°C	16 min	4 hours	2 hours

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2.2 Organoleptic characters and classical bhasma parīkṣa.

Organoleptic characters include colour, odour, taste, and state. Classical bhasma parīkṣas done were Vārītaratwa, Rekhāpūrṇatwa, Niścandratva, Unnama, Dadhi parīkṣa.[6]

Vārītaratwa: A pinch of prepared bhasma is put over stable water in a bowl, ideal bhasma should float over the surface of the water.

Rekhāpūrṇatwa: This test is done to ascertain the fineness of the bhasma. Here a pinch of bhasma is rubbed between the thumb and index finger. If the bhasma enters and embeds into the fingerprints, it indicates Rekhāpūrṇatwa.

Niścandratva: A little quantity of the bhasma was rubbed between the thumb and index finger and observed under sunlight, to check the shining particles, no shining particles were found during the test.

Unnama: A pinch of prepared bhasma is put over stable water in a bowl and a rice grain was placed over the bhasma. The rice particle was floating on the surface of water and did not sink to the bottom.

Dadhi pareeksha: To test the perfectness of Tāmra bhasma, a pinch of Tāmra bhasma is added to a bowl containing Dadhi and left for 24 hours. If the Dadhi didn't exhibit bluish shade, then the bhasma is appropriately prepared.

2.3 Physico-chemical parameters

Parameters done were pH (1% aqueous solution), LOD at 110◦C, Total ash, Acid insoluble ash, Sulphated ash. The procedure was done according to Ayurvedic Pharmacopeia of India.7

2.4 Advanced instrumental analysis

2.4.1 Dynamic light scattering and Zeta potential

Zetasizer Nano ZS was used for particle size analysis and zeta potential and water as the liquid media.

2.4.2 Elemental analysis by ICP-OES

Optima 5300DV was used for analysis. A known quantity of the solid sample was digested in acid mixture and diluted to a known volume and analysed as per the work procedure for ICP-OES analysis. The concentration of the element in the solution was determined from the calibration plot obtained by analysing standard solutions. The results were recorded and processed using Win Lab 32 software.

2.4.3 Morphology by Scanning electron microscopy and energy dispersive spectroscopy

The SEM images of the sample were taken by Zeiss EVO 18 Scanning Electron Microscope, with magnifications at 10.00 K X, 15.00 K X, 25.00 K X, 35.00 K X and 45.00 k X at a resolution of 100 nm,200 nm, 1 µm and 2 µm. For SEM analysis, a very small portion of your sample is taken on a sample holder and kept for sputter coating for 3 to 5 minutes. Au-Pd alloy was used as the sputter coating target. Then sample is loaded to scanning electron microscope. Image capturing is done with secondary electron detector.

2.4.4 Phase identification by X-ray Diffraction

XRD patterns were obtained using MiniFlex 600 X-ray diffractometer with 40 KV voltages and 15 MA current. Software used was SmartLab Studio II.

2.4.5 In vitro study

1. Cytotoxicity studies by MTT assay

Preparation of test material for MTT assay

Test samples was steam sterilized at 121°C for 20 min. Extract was prepared dissolving test samples TB and TBA in 5% DMSO at concentration of 2 mg/ml and diluted with MEM 2X (Minimum Essential Medium) to get concentration of 1 mg/ml considered as 100%. The samples were then incubated 37±1°C for 72±2 hours.

Preparation of reagent control

5% DMSO diluted with MEM 2X and incubated at 37°C.

Preparation of positive control

Positive control was prepared by diluting phenol stock solution (13 mg/ml with culture medium containing serum).

Procedure of cytotoxicity by MTT assay

The MTT assay was performed to measure the metabolic activity of cells to reduce yellow coloured tetrazolium salt 3-(4, 5- Dimethyl thiazol- 2-yl)-2,5-diphenyltetrazolium bromide to purple coloured formazan. Extract was prepared dissolving test samples TB and TBA in 5% DMSO at a concentration of 2 mg/ml and diluted with MEM 2X to get concentration of 1 mg/ml which is considered as 100%. The samples are then incubated 37±1°C for 72±2 hours.

After 72 hours the extract was diluted with culture medium to get 50%, 25%, 12.5% and 6.25%. Cells

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cultured in normal medium was considered as cell control. 5% DMSO diluted with MEM 2X was considered as reagent control. Equal volume (100µl) of various dilution of test samples, extract of reagent control, cell control and positive control were placed on subconfluent monolayer of L929 cells. After incubation of cells with various concentration of test sample and controls 37±1°C for 24±2 hours, extract and control medium were replaced with 50µl MTT solution (1 mg/ml in medium without supplements), wrapped with aluminium foil and were incubated at 37±2 hours. After discarding the MTT solution 100µl of Isopropanol was added to all wells and swayed the plates. The colour developed was quantified by measuring absorbance at 570 nm using a spectrophotometer. The data obtained for test samples were compared with cell control.

2. Blood compatibility studies.

Exposure of materials with blood

Blood from human volunteer was collected into the anticoagulant, ACD (Anticoagulant Citrate Dextrose Solution). The two samples were submitted (1 mg each in the powder form) by the customer. Each test material was added to 4 ml of blood in polystyrene petri dishes to get a final to get a final concentration of 0.25 mg/ml. 2 ml blood was taken immediately for initial analysis and remaining 2 ml blood was incubated with the samples for 30 min under agitation at 70±5 rpm using an Environ shaker thermostat at 35±2˚C (WPTRU012). Three empty polystyrene tubes were exposed with blood as reference.

Preparation of Platelet Rich Plasma (PRP)

The PRP was prepared as per WPTRU005. The blood sample was centrifuged at 200g for 5 min and PRP was aspirated.

Preparation for Platelet Poor Plasma (PPP)

The blood samples were centrifuged at 2500g for 15 min as per WPTRU006 and platelet poor plasma (PPP) was aspirated.

a) Hematology

i. Plasma haemoglobin

The free haemoglobin liberated into the plasma after exposure to samples was measured using Diode Array Spectrophotometer as per WPTRU022 and the percentage change was calculated.

ii. Percentage hemolysis

The total haemoglobin in the whole blood samples were measured using automatic

Hematology Analyzer (ERBA Mannheim- H360) as per WPTRU015. The free haemoglobin liberated into the plasma after exposure to samples was measured using Diode array Spectrophotometer as per WPTRU022 and the percentage hemolysis was calculated using the formula (Free Hb/ Total Hb/1000)x 100.

b) Quantification of platelet aggregates

The platelet counts in blood were analysed in initial and 30 min samples using Hematology Analyzer (ERBA Mannheim- H360) as per WPTRU015. The equipment calibration was verified using internal control.

c) Coagulation

i. Partial Thromboplastin Time (PTT)

Partial Thromboplastin time in initial and 30 min plasma samples were detected using a reagent kit obtained from Diagnostica Stago (France) on Start 4, coagulation analyzer as per WPTRU023.

ii. Fibrinogen

Clottable fibrinogen in initial and after 3o min exposed samples were measured using a reagent kit obtained from Diagnostica Stago (France) on Start 4, coagulation analyzer as per WPTRU026.

d) Platelet Activation- Estimation of P-selectin

P-selectin was analysed by flowcytometry as per WPTRUO59. Briefly the samples were diluted with ACD-PBS to get final Platelet count of 150-300 x 10^4 ml $^{-1}$. The samples were then treated with fluorescent tagged (PE) CD62P antibody and after 1 hour incubation diluted with PBS and analysed using a flow cytometer (CytoFLEX Beckman Coulter)

In the present study, Organoleptic and physio-chemical analysis of Tāmra bhasma with and without amrİtīkarana was done in attached Quality control laboratory, Dept. of Rasaśāstra and Bhaisajya Kalpana, Amrita School of Āyurveda, Kollam. Particle size distribution, Zeta potential, ICP-OES, in vitro cytotoxicity and blood compatibility studies was done at Sree Chitra Tirunal Institute for Medical Sciences & Technology, Thiruvananthapuram. SEM-EDS was done at CSIR-NATIONAL INSTITUTE FOR INTERDISCIPLINARY SCIENCE & TECHNOLOGY, Ministry of Science and Technology, Govt. of India Thiruvananthapuram. XRD was done at IR Technology Services Pvt Ltd, Navi Mumbai.

3. Results

Both samples of TB and TBA had passed the classical bhasma parīkṣas like Vārītaratwa, Rekhāpūrṇatwa, Niścandratva, Unnama, Dadhi parīkṣa. Organoleptic parameters and physico-chemical parameters of TB and TBA are shown in Table 2.

Table 2 showing organoleptic characters and physico-chemical parameters of TB and TBA

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Sl No	Parameters	Observations	
		TB	TBA
1	Colour	Black	Dull black
2	Odour	No characteristic odour	No characteristi odour
3	State	Powder	Powder
4	Taste	No characteristic taste	No characteristi taste
5	pH (1% aqueous solution)	5.77 at 27.4°C	4.69 at 27.4°C
6	Total ash	76.354% w/w	47.846% w/w
7	Acid insoluble ash	2.955% w/w	2.39% w/w
8	Sulphated ash	60.51% w/w	30.31% w/w
9	Loss on drying at 110°C	0.55% w/w	3.55% w/w

The particle size distribution of TB and TBA are shown in figures 1 and 2. TB had a mean particle diameter of 1378 nm and TBA had mean particle diameter of 556.4 nm. Zeta potential of TB are shown in figure 3 and TBA are shown in figure 4. Zeta potential of TB and TBA are -3.04 and -13.7 respectively. Elemental composition of TB and TBA are shown in table 3 and XRD results of TB and TBA are shown in table 4 and in graph 1 and 2. SEM images of TB and TBA are shown in figure 5 and 6. EDS results of TB and TBA are shown in tables 5. Results of in vitro analysis of TB and TBA are shown in table 7 and 8. MTT Assay results of TB and TBA are shown in graph 3

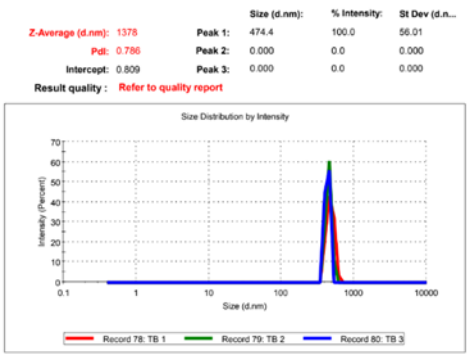

Figure 1: Showing particle size distribution of TB

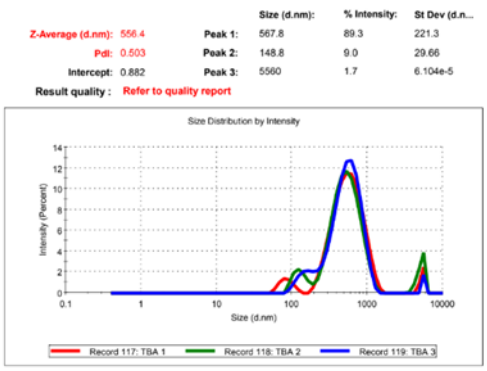

Figure 2: Showing particle size distribution of TBA

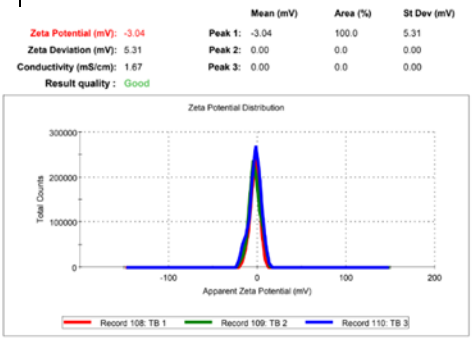

Figure 3 showing zeta potential of TB

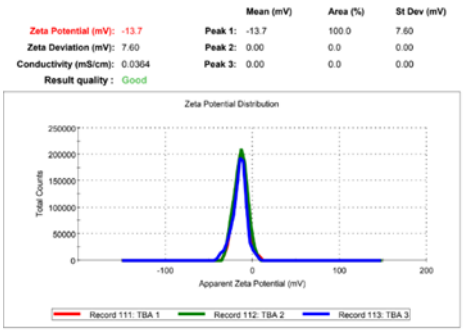

Figure 4 showing zeta potential of TBA

Sl No	Parameters	*Tāmra bhasma* without *amrĪtīkarana*	*Tāmra bhasma* with *amrĪtīkarana*
1	Copper	56.44%	24.18%
2	Mercury	0.30%	0.02%
3	Arsenic	21.31%	7.87%
4	Sulphur	15.44%	11.91%

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5	Iron	0.30%	0.20%

Table 3 showing results of ICP-OES of TB and TBA

Table 4 showing results of XRD of TB and TBA

XRD		XRD	
TB		TBA	
2θ	**Counts**	**2θ**	**Counts**
21.180	1143	12.380	181
30.080	4945	21.440	536
		24.780	297
		27.800	1013
		30.4400	15991
		32.940	626
		35.280	2246
		37.500	887
		41.80	159
		43.520	207
		45.440	970
		48.980	1681
		50.720	6738
		52.360	346
		54.00	148
		55.640	660
		57.16	140
		60.280	2985
		63.22	296
		64.68	354
		67.60	332
		68.92	92
		74.50	605
		78.58	159

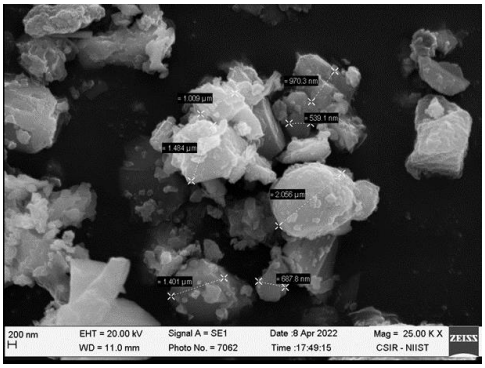

Figure 5 showing SEM images of TB

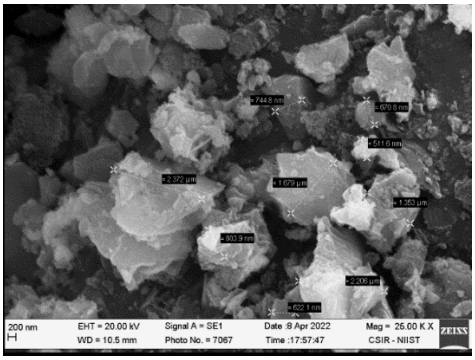

Figure 6 showing SEM images of TBA.

Table 5 showing EDS result of TB and TBA

	TB		TBA	
	Weight %	Atomic %	Weight %	Atomic %
Sulphur	30.13	47.99	60.58	76.06
Copper	48.51	39.88	28.69	18.18
Arsenic	17.75	12.10	10.72	5.76
Mercury	3.61	0.92	Not detected	Not detected

Table 6 showing Cytotoxicity experiments by MTT assay at 72±2 hours

CONCENTRATION	TB	TBA
100%	61.55%	47.28%
50%	96.14%	47.76%
25%	108.10%	75.15%
12.50%	109.71%	87.96%
6.25%	114.31%	97.53%

Table 7 showing Blood compatibility studies of TB and TBA

		TB	TBA
Plasma haemoglobin	Plasma Hb Initial (mg/dL)	5.29	5.59
	Plasma Hb Final (mg/dL)	6.06	6.10

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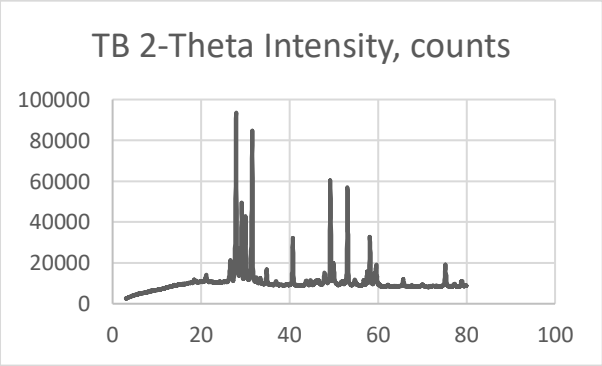
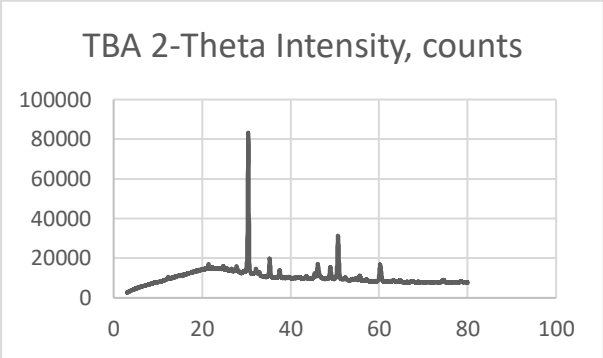
	Percentage change	14.51	9.08
Percentage hemolysis		0.04%	0.04%
Platelet count	Initial x10^8 cells/ml	1.5	1.53
	Final x10^8 cells/ml	1.41	1.37
	Percentage change	6	10.46
Partial thromboplastin time	Initial PTT (sec)	74.9	77.7
	Final PTT (sec)	73.6	73.2
	Percentage change	1.74	5.79
Clottable fibrinogen	Initial PTT (g/L)	3.19	2.99
	Final PTT (g/L)	3.15	3.19
	Percentage change	1.25	6.69
P- Selectin	% of activated platelets (Initial)	2.40	2.70
	% of activated platelets (Final)	0.96	2.64
	Percentage change	1.44	0.05

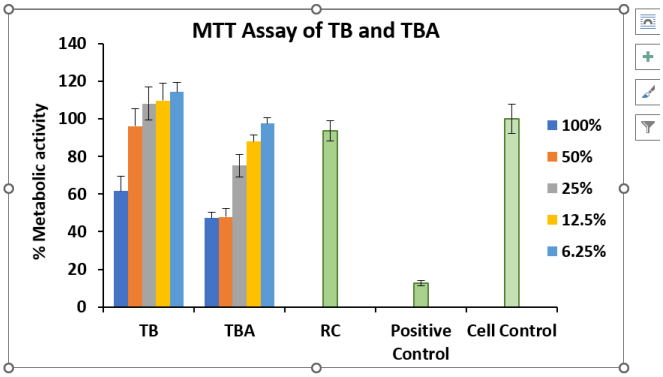
Graph 1Showing XRD pattern of TB



Graph 2 Showing XRD pattern of TBA



Graph 3 Showing MTT Assay results of TB and TBA



4. Discussion

4.1 Discussion on Pharmaceutical study

4.1.1 Sāmānya śodhana

Hissing sound along with vapours were produced on dipping the red hot Tāmra patra into the media. When the red hot Tāmra patra was immersed in tila taila, flame was seen for few seconds and irritant fumes were released simultaneously. After each quenching, Tāmra was found dull, and some soot was adhered on the surface of the patra and some remnant in the form of powder was observed. The amount of sediment was found maximum (average 42g) in kulattha kvātha. Weight gain was observed after quenching with tila taila. This may be due the adhesion of tila taila particles that could not be removed even after thorough washing with hot water. Tāmra patra became more brittle and changed to blackish red after the samanya śodhana.

4.1.2 Viśeṣa śodhana

After 10 min of boiling, froth was observed on gomutra and colour turned to blackish green with a strong irritating odour. After boiling, colour of Tāmra changed to greenish colour. Some particles remained at the bottom of the vessel with the liquid media. These

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particles were carefully collected from the liquid medium. Ammonia present in gomutra can react with copper to form the chemical complex cuprammonium ion [Cu $(NH_3)_4$]. It can get removed on washing with hot water. The weight loss which was observed after viśeṣa śodhana may be a result from loss of this newly formed complex.[9]

4.2 Discussion on analytical study

In this study, after classical bhasma parīkṣa both samples of Tāmra bhasma was subjected to basic physicochemical analysis for quality assurance. The value of loss on drying in both samples indicates absence of moisture content. LOD was less in TB compared to TBA. Even though pH of both samples are slightly acidic, TBA was found to be more acidic. High ash value in TB shows the presence of high inorganic content. Ash value is less in TBA compared to TB, which may be due to the addition of Paňcāmrİta during its preparation. Lower acid insoluble ash value of both samples suggests the greater bioavailability of the drug. Sulphated ash value of TBA is less compared to TB, which indicates the presence of more sulphates in TBA.

4.3 Discussion on advanced instrumental analysis

DLS results shows that the particle size of Tāmra bhasma was reduced after amrİtīkarana. The results suggests that the sample is in polydisperse state. Polydisperse means non-uniformity of size distribution of particles. Zeta potential of TB and TBA shows that TBA sample is more stable than TB.

In ICP-OES, the percentage composition of both samples of Tāmra bhasma was analysed. Elemental analysis by ICP-OES results shows that mercury content in both samples were found to be within the limit. Arsenic content was reduced in TBA sample comparing to TB. Comparing to TB, all the elements were reduced in TBA, this may be due to extra 3 putas in TBA.

SEM images of 25K magnification reveal that the size of the particles ranges from nanometer to micrometer level. The particle size range of TB is 539.1 nm to 970.3 nm and 511.6 nm to 803.9 nm for TBA. Agglomerates were also visualized. The EDS pattern at three surface sites are also given, which supports the ICP-OES results as well.

XRD analysis was used to identify the crystalline phases of the samples and to estimate their respective crystallite sizes. The XRD results of TB sample shows characteristic peaks at 2θ values of 13.92, 18.42, 21.180, 22.040, 26.660, 27.940, 28.26, 28.880, 29.180, 30.080, 30.220, 31.560, 32.50, 33.420, 34.820, 36.960, 37.98, 40.04, 40.740, 43.820, 44.800 of which the peaks at 21.180, 30.080 corresponds to $Cu_{12}As_4A_{13}$ in Cubic crystalline form. XRD results of TBA sample shows characteristic peaks at 2θ values of 12.380, 14.54, 21.440, 24.780, 25.54, 26.700, 27.800, 29.50, 30.4400, 32.200, 32.940, 35.280, 37.500, 41.40, 43.520, 45.440, 46.140, 48.980, 50.720, 52.360, 54.000, 54.680, 57.16, 60.280, 63.22, 64.68, 67.60, 68.92 corresponds to $Cu_{12}As_4A_{13}$ in Cubic crystalline form, the remaining 2θ values are attributed to the presence of Cu_9S_5 in Rhombohedral crystalline form. The formation of sulphates after amrİtīkarana can prove analytically that it will be less toxic. The sample TBA with CuS will be safer rather than TB in elemental form.

4.4 Discussion on Invitro Study

4.4.1 Cytotoxicity experiments

The MTT assay of L929 cells after contact with extracts of 1mg/ml, 0.50 mg/ml, 0.25 mg/ml, 0.125 mg/ml, 0.0625 mg/ml at 72±2 hours shows that both samples are non- cytotoxic. Maximum and minimum cell activity was seen at concentration 0.0625 mg/ml and 1 mg/ml for both samples. Both samples showed cytocompatibility at lower concentrations. TB cells shows slightly increase viability at larger concentration.

4.4.2 Blood compatibility studies

The hemolysis induced by TB and TBA was 0.04% which was well within the acceptable limits of 1%. Based on hemolysis value we can assume that plasma haemoglobin level of samples are also normal. Change in values of platelet count was shown in both samples. Comparing to TB, TBA shows more variation. Measurement of uncertainty acceptable value is 5% but here, 10% change is shown for sample TBA. This shows that TBA encourages platelet adhesion so, this can be used in bleeding disorders. The control plasma and the plasma containing the TB sample shows almost equal amounts of fibrinogen and partial thromboplastin time. But TBA sample shows increased value in results comparing to the control. Despite the observed difference, no delay in coagulation time was observed in plasma samples. Activation of platelets initiates the deformation of the cells with pseudopod formation and ends with blood coagulation or thrombus formation. In the present study platelets seem to be not activating indicates platelet compatibility of both samples.

5. Conclusion

For providing necessary scientific validation for structure and composition, of Tāmra bhasma prepared with and without amritīkarana, physico-chemical parameters and advanced instrumental analysis were done. Both samples of Tāmra bhasma have particles ranging from nanometer to micrometer level. For providing necessary scientific validation for structure,

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composition, of Tāmra bhasma prepared with and without amritīkarana, physico-chemical parameters and advanced instrumental analysis were done. Both samples of Tāmra bhasma have particles ranging from nanometer to micrometer level. From this study it is evident that amritīkarana helps in making Tāmra bhasma safer based on the physio-chemical parameters. As per methodology, Tāmra bhasma prepared without amritīkarana showed better results in blood compatibility.

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