Published online in http://ijam.co.in ISSN No: 0976-5921

International Journal of Ayurvedic Medicine, Vol 13 (2), 487-494

Characterization of Copper-based Ayurved Medicine *Tamra bhasma* produced by various manufacturers and its Pharmacokinetic profiling in Wistar rat

Research Article

Chaitali S Waghmare[1], Shivcharan Bidve[1]*, Gudi R V[2], Mukesh B Chawda[3], Santosh Yadav[2]

1. Shree Dhootapapeshwar Ayurvedic Research Foundation, Veer Savarkar Chowk, Panvel, Maharashtra, India.
2. Shree Dhootapapeshwar Limited, Veer Savarkar Chowk, Panvel, Maharashtra, India.
3. Solumiks Herbaceutical Limited, Fort, Mumbai, Maharashtra, India.

Abstract

Background: *Tamra bhasma* (TB) is copper based herbo-metallic preparation which is used extensively by Ayurvedic practitioners. *Tamra bhasma* is endorsed for different disorders of liver, abdominal pain, heart disease, colitis, tumors, anemia, loss of appetite, tuberculosis, as well as eye problems. Objective: Our aim is to characterize 5 commercial TB preparations from 5 different manufacturers by using modern scientific techniques and to study there bioavailability in Wistar rats. Materials and Methods: *Tamra bhasma* was characterized by X-ray diffraction (XRD), Scanning electron microscope (SEM), Energy Dispersive X-ray analysis (EDAX), Nanoparticle tracking analyzer (NTA), Inductively coupled plasma optical emission spectroscopy (ICP-OES). Bioavailability of *Tamra bhasma* was studies using non compartmental rat model with daily dose of 6.45mg/kg according to their body weight. Results: The colour of one of the TB preparation was different from other 4 TB samples. The chemical phase and particle size is significantly different for all the 5 TB's. Pharmacokinetic model confirms difference in various PK parameters such as peak concentration (C_{max}), half-life ($t_{1/2}$) and terminal elimination slope (λ_z) for all 5 TB's. TB-A showed highest C_{max} (82.21 mg/L), whereas TB-E showed lowest C_{max} (48.69 mg/L). The highest bioavailability of TB is may be due to specific chemical moiety and morphology. Based on XRD and elemental analysis, it was found that manufacturing route followed for one of the preparation is not as per ayurvedic text reference. Conclusions: The morphology as well as chemical phase of the five TB's studied were different from each other, which might be responsible for different pharmacokinetic profiles in Wistar rat model.

Key Words: *Ayurveda, Tamra bhasma,* Pharmacokinetic, XRD, NTA, Wistar rat.

Introduction

Ayurveda is well known for use of naturally occurring minerals and herbo-mineral preparations to cure many diseases since centuries. Naturally occurring metals and minerals were converted into *bhasma* (calcined powder) by following procedures like, *shodhana* (purification or detoxification), *marana* (incineration or calcination), *amritikarana* etc (1). The prepared *bhasmas* are highly effective without any untoward effects in the therapeutic dose. *bhasmas* are characteristic preparations that are being practiced safely in Ayurveda without any noticeable side effects (2, 3).

Ayurvedic herbo metallic drug *Tamra bhasma* (TB) is prepared from metallic copper which is used extensively for the treatment of *pandu* (anemia), *udara* (ascites), *svasa* (asthama) and *amlapitta* (hyperacidity), liver disorders, leucoderma, etc (4-8).

Copper deficiency in the body can lead to problems with weight loss, connective tissue, muscle weakness, microcytic hypochromic anemia, low white blood cell count, graying of hair, neurological problems, and paleness (9-11). The safety and efficacy of a *Tamra bhasma* depend on the classical ayurvedic text reference followed for its preparation. Any deviation from the Ayurvedic text reference preparation method will not yield desired results. Earlier, safety of *Tamra bhasma* was demonstrated by different research groups like Chaudhari et al. (3), Vahalia et al. (12), Jagtap et al. (13, 14), Mohan et al. (15) etc.

In recent times efficacy of *Tamra bhasma* on lipid profile in albino rats were reported by Dabikar et al. (16). Jagtap et al. have reported that *Tamra bhasma* prepared from *shodhit Tamra* is having significant anti-hyperlipidemic activity and *ashodita Tamra bhasma* possess cardio-toxic effect (13). Free radical scavenging activity of *Tamra bhasma* was studied by Pattanaik et. al. (7). Patil et al. have studied the effect of *Tamra bhasma* on lipases and lypolitic activities in CCl_4 induced hepatic injury in rats (17).

*** Corresponding Author:**

Shivcharan Bidve

QC Officer,
Shree Dhootapapeshwar Ayurvedic Research Foundation, Veer Savarkar Chowk, Panvel, Maharashtra, India.
Email Id: shiv1925bidve@gmail.com

In India, Shree Dhootapapeshwar Limited, Baidyanath Ayurved Bhawan Pvt. Ltd., Unjha Pharmaceuticals Pvt. Ltd., Krishna Gopal Ayurved Bhavan and Divya Pharmacy are amongst well known large scale manufacturers of TB. Manufacturing route used to prepare TB by different manufacturers may vary as per the textual method followed. The market price for 5g of TB varies from Rs 200 to Rs 400. TB characterized by different research group confirms different chemical moiety which depends on different textual reference followed for its preparation. Singh et al. (18) and Wadekar et al. (19) reported Cupric oxide as main phase for prepared TB. Chaudhari et al (20) confirms presence of CuS and free Sulphur for prepared TB. Not only morphology but the presence of specific chemical moiety may be responsible for its bioavailability. Therefore it is very important to study pharmacokinetic activity of *Tamra bhasma* manufactured with reference to different textual methods.

Bioavailability is an important pharmacokinetic property of therapeutic agent that defined the fraction of administered dose of unchanged drug which reaches the systemic circulation. *Bhasma* particles after oral dose could be available in the blood circulatory system and can accumulate in various tissues. Bio availability of *bhasma* is depend on many factors such as particle size, particle shape, chemical phase etc. In current study we have demonstrated the concentration profile of Copper (Cu) in serum with time in the Wistar rat model after oral administration of similar dose of TB with different preparations.

Materials and methods

Chemicals

Tamra bhasma used for study was manufactured by Shree Dhootapapeshwar Ltd. (SDL), Panvel, Navi Mumbai, India. The different competitors used for the study was procured from Ayurvedic chemist shop. The manufacturing details of TB's were compiled in **Table 1**.

Characterization of *Tamra bhasma* (TB)

Tamra bhasma (TB) was characterized to understand its physicochemical properties. The structural and phase details of TB were analysed by powder X-ray diffractometer (XRD, MiniFlex 600, Rigaku, Japan) and peaks were compared with ICDD PDF-2 2021 (International Center for Diffraction Data). Scanning electron microscope with EDAX (SEM) (JEOL, Japan) was used to observe morphology and elemental composition. Copper (Cu) concentration in serum was analyzed by Inductively coupled plasma optical emission spectroscopy (ICP-OES, Avio-200 Perkin Elmer, USA). Particle size of TB was determined by Nanoparticle Tracking Analyzer (NTA, NS300, Malvern Panalytical, UK).

Animals used

A total of thirty healthy Male Wistar rats (180-220 g) were procured from the In-House Animal Facility of Shree Dhootapapeshwar Ayurvedic Research Foundation (SDARF). The weight variation of animals used did not exceed ± 20 % of the mean weight. Animals were provided with standard diet and water *ad libitum*. Animals were housed in plastic cages below Standard conditions, temperature 20 ± 2°C and humidity 30-70%, with 12 h dark/light cycle. Maximum three animals were housed in a single cage. The Animals were acclimatized for a minimum period of seven days prior to exposure of test items.

Animal ethics

The experimental protocol was approved by the Institutional Animal Ethics Committee of SDARF (study number: SDARF/2020/01). The experiments were conducted according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

Experimental Design

The animals were randomly divided into group of five, each group contain six animals (Table 2). Group I received *Tamra bhasma* of SDL with honey and lukewarm water at dose of 6.45mg/kg according to their body weight. Remaining all four groups received *Tamra bhasma* of different competitors with honey and lukewarm water at dose of 6.45mg/kg according to their body weight.

Bioavailability study

All animals were fasted for 4-6 hours prior to dosing (Feed of animals withheld. Animals were allowed free access of water). Initially blood was collected at zero hour from all animals. All animals were orally gavaged with *Tamra bhasma* mixed with honey and lukewarm water. After administration of drug, blood was collected at interval of 2, 4, 8, 24 & 48, 54, 72 hours. 1 ml of blood was collected at each time. The blood was kept at room temperature to clot. The clotted blood was separated by centrifugation to separate serum. The serum was analyzed for the copper content by using ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry).

Estimation of Copper content in serum

The collected serum was kept on water bath for evaporation of water content. The serum sample is transferred to crucible for incineration in muffle furnace at 600^{0}C for 10-20 mins to form carbon free Ash. 10 ml Aqua regia (HNO_3: HCL in 1:3 ratio) is added to the ash sample. This mixture was kept for digestion, followed by filtration after complete digestion. The Filtered sample was further used to analyze Copper (Cu) content on ICP-OES.

Statistical Analysis

PK Solver software was used to calculate various PK parameters such as time of peak concentration (Tmax), terminal elimination slope (λ_z), area under the zero and first moment curves from 0 to last time t (AUC_{0-t}, $AUMC_{0-t}$), half-life ($t_{1/2}$), mean residence time (MRT) and apparent volume of distribution based on the terminal slope ($V_{z/F}$).

Results

XRD analysis

XRD peaks of TB-A, TB-B, TB-C, TB-D and TB-E were different as shown in **Fig. 1**. Data obtained from XRD analysis such as crystallite size, phase present, DB card number and crystal system of TB was presented in **Table 3**. XRD of sample TB-A shows presence of CuS extensively and small amount of free sulphur. XRD of TB-B confirms presence of CuS, free sulphur and $CuSO_4.5H_2O$. TB-C sample shows presence of CuO extensively with small amount of $Cu_2Cl(OH)_2$. TB-D confirms presence of Fe_2O_3 (Hematite) and Cu_2S (chalcocite) mainly. XRD of TB-E shows presence of CuO only. The crystallites size calculated using Debye-Scherrer formula and found to be maximum for TB-D (67.6 nm), whereas, the other four samples had crystallites size in-between 44 to 50nm.

SEM-EDAX analysis

SEM-EDAX analysis results were presented in Fig. 2 and Table 4. SEM report confirms that TB-A sample were flakes shape and EDAX analysis shows presence of Cu, S as major element and Fe in trace amount. TB-B sample is mixture of spherical particles as well as flakes and EDAX analysis confirms presence of Cu, S, Cl and O. TB-C sample confirms agglomerated spherical shape particles with presence of Cu, S, Fe, Cl and O. TB-D sample consist of mixture of flakes shape and agglomerated spherical shape particles with presence of Fe, O mainly and traces of Cu and S. TB-E sample consist of agglomerated spherical structural morphology with presence of Cu, S, Fe and O.

EDAX analysis of TB preparations revealed some stimulating facts about these samples (Table 4). In sample TB-D, Copper content was found minimum and Iron content was maximum.

NTA analysis

Nanoparticle tracking analysis (NTA) results were presented in Table 5 and Fig. 3. TB-A sample confirms particle size varies from 15nm to 100 nm and average particle size of the TB-A was found to be 55nm. 50% particle are below 50nm size. TB-B sample NTA graph shows that particle size varies from 6nm to 100nm with mean particle size of 43nm. Particle size for TB-C sample varies from 5nm to 150nm and average particle size was found to be 49nm. TB-D sample particle size varies from 9nm to 160nm and mean particle size was found to be 47nm. Sample size for TB-E sample varies from 10nm to 120nm and average particle size was found to be 53nm.

Bioavailability of Copper in Wistar rat serum

The concentration of *Tamra bhasma* in serum at different time intervals after oral administration of drug in Wistar rats were shown in Table 6 and Fig. 4. After oral administration the maximum concentration i.e. Cmax of TB-A in serum was found to be 82.2 ppm. The Tmax in TB-A sample as in other competitor sample was shown at 48 hours. Statistical analysis revealed that there is an increase in the Concentration of Copper content in TB-A sample compared with other competitors after drug administration.

The schematic of the Wistar rat experiment is demonstrated in Fig 4. Bioavailability of Copper (Cu) in serum was established by measuring the time-dependent content of copper in the serum (Fig 4). The concentration-time profile of TB-C was largely distinct from the other 4 samples. Tmax for all TB samples was at 48 hours after drug administration. The non-compartmental pharmacokinetic model (Table 6) showed a significant difference in λz, T_{max}, C_{max}, AUC_{0-t}, and V_z/F_{obs} parameters between various TB groups. TB-A showed the highest C_{max} copper concentration after 48 hours of drug administration among all the TB samples and SB-E showed the lowest.

Discussion

Metals and minerals are very important part of Ayurvedic formulations since centuries. The manufacturing process of *bhasma* is tedious and involved steps like *shodhana*, *marana* etc. Final step *amritikarana* is used to remove any possible impurities remain in *bhasma* after *marana* process (21). However, due to high demand many Ayurvedic drug manufacturers do not follow all steps stated in Ayurvedic text references. These malpractices could lead to toxic effects instead of its benefits.

The time saving route or shortcuts followed for *bhasma* preparations could lead to variation in physicochemical parameters and may also form different chemical moiety than expected, which can lead to serious ill effects to patient. Efficacy and pharmacokinetic availability of any drug is dependent on physicochemical properties such as shape, size, composition and chemical moiety of that particular drug.

Hence, to prove quality and efficacy of these Ayurvedic herbo mineral medicines, physicochemical characterization is of utmost important parameter. Also, it is very much important to validate batch to batch consistency of any Ayurvedic drug as a quality parameter.

Our study found variations in physicochemical properties in marketed TB samples prepared using different textual references. Very first point to note that general appearance i.e. colour for 4 TB are almost same and one of TB sample i.e. TB-D colour is different, which could be due to different chemical moiety. The chemical phase for all 5 preparations was different.

TB-A confirms presence of CuS and free sulphur. TB-B consisted of CuS, $CuSO_4.5H_2O$ and free sulphur. Whereas TB-C consist of CuO and $Cu_2Cl(OH)_3$. TB-E sample is mainly consisted of CuO. TB-D sample found to be suspicious as it confirms presence of Fe_2O_3 as major phase and Cu_2S as minor phase. Hence, it might be possible that TB-D sample is not prepared as per ayurvedic textual reference.

The chemical composition and morphology of all 5 TB preparations was different. As demonstrated by earlier researchers, particle size and shape control for

bhasma preparations is quite challenging after following typical Ayurvedic process. Particle size distribution is mostly wide (from nm to um) and particles are irregular in shape. In this study we observed that all the samples are either flakes shape or agglomerated spherical shape. TB- D sample consist of iron as major element and copper as minor element, which makes it brownish in appearance. It could be concluded that TB-D was probably not prepared by Ayurvedic text reference.

To know the efficacy of TB in terms of pharmacokinetic (PK) properties, bioavailability of copper should be investigated. Absorption of *bhasma* can be affected by morphology of particle as well as chemical moiety present. In this Wistar rat model experiment, we observed that pharmacokinetic parameters for all 5TB preparations are different with respect to the maximum copper absorption in the serum (C_{max}). This variation in C_{max} could be due to physicochemical properties of TB.

The maximum concentration was found at 48 hours after oral administration. The TB-A sample shows maximum absorption of drug in the systemic circulation as compared with other competitors. There was an increase in the availability of drug in serum shown by TB-A sample. The other competitor also shows maximum availability at 48 hours after oral administration.

The observed variation in bioavailability could be due to variation in physicochemical parameters such as particle size, shape and chemical moiety present. Herein we observed that TB-A which have CuS as major phase shows highest bioavailability as compared to other TB preparations which possesses CuO, Fe_2O_3 as main chemical moieties.

Conclusion

Current study investigates the difference in *Tamra bhasma* prepared by 5 different manufacturers with different morphology as well as chemical moiety. One of the samples contain trace amount of copper and mainly consist of iron, which is suspected not be prepared as per the claimed Ayurvedic textual references. We have demonstrated that the morphology as well as chemical phase of the five TB's studied were different from each other, which might be responsible for different pharmacokinetic profiles in Wistar rat model. Hence this study shows that efficacy of different TB manufacturers may vary and suggest stringent control over the manufacturing process and quality of TB.

Funding: This research was funded Shree Dhootapapeshwar Limited, Mumbai, India.

Declaration of interest: The authors declare that there are no conflicts of interest.

Acknowledgement

Author would like to thanks Dr. Rajesh Raut, Botany Department, The Institute of Science, Mumbai for providing NTA facility.

Tables

Table 1: TB prepared using different textual references

Tamra bhasma (sample code)	Date of manu-facturing	Textual manufacturing reference as per label	Batch no.
TB-A	Mar-20	*Rasaratna Samuchaya 5/53*	P200300080
TB-B	May-18	*Siddhayog Sangrah*	140122
TB-C	Jan-19	Ayurvedic Formulary of India. part I	#B TMB007
TB-D	Jun-18	*Bharat Bhaishajya Ratnakar 2582*	2
TB-E	Nov-19	*Rasatantrasar & Siddhaprayogsangrah (1st)*	01/11

Table 2: Grouping of Wistar rats and dose level of *Tamra bhasma* for different competitors

Group no.	Group	Dose (mg/kg body weight)	No of males	Time interval for blood collection
I	TB-A	6.45	6	0, 2, 4, 8, 24, 48, 56, 72 hr
II	TB-B	6.45	6	
III	TB-C	6.45	6	
IV	TB-D	6.45	6	
V	TB-E	6.45	6	

Table 3: Color and XRD data for TB prepared using different text reference

Sample	Crystallite size (nm)	Phase	DB Card number	Crystal system	Space group	Colour
TB-A	50.3	Covellite (CuS)	01-078-0877	Hexagonal	194: P63/mmc	Black
		Sulphur (S8)	01-078-1888	Orthorhombic	70: Fddd:2	
TB-B	44.5	Covellite (CuS)	01-078-0877	Hexagonal	194: P63/mmc	Black
		Sulphur (S8)	01-078-1888	Orthorhombic	70: Fddd:2	
		Copper sulphate pentahydrate (CuSO4 (H2O)5)	01-077-1900	Triclinic	2:P-1	
TB-C	46.0	Copper Oxide (CuO)	01-080-1913	Monoclinic	9:C1c1	Black
		Atacamite (Cu2Cl(OH)3)	01-080-9252	Orthorhombic	62: Pnma	
TB-D	67.6	Hematite (Fe2O3)	01-080-2377	Trigonal	167: R-3c:H	Brown
		Chalcocite (Cu2S)	01-078-4793	Hexagonal	194: P63/mmc	
TB-E	46.6	Copper Oxide (CuO)	01-080-1916	Monoclinic	9:C1c1	Black

International Journal of Ayurvedic Medicine, Vol 13 (2), 487-494

Table 4: EDAX analysis results for TB prepared with different textual references

Element (At %)	TB-A	TB-B	TB-C	TB-D	TB-E
Oxygen (O)	----	26.69 ± 3.05	50.93 ± 3.51	53.71 ± 3.08	49.97 ± 3.17
Sulphur (S)	46.13 ± 2.36	28.37 ± 1.75	1.67 ± 0.45	0.90 ± 0.35	0.45 ± 0.29
Chlorine (Cl)	ND	0.61 ± 0.31	4.02 ± 0.64	ND	ND
Iron (Fe)	2.06 ± 0.56	0.19 ± 0.22	0.27 ± 0.24	44.48 ± 2.24	7.52 ± 0.85
Copper (Cu)	51.81 ± 3.35	44.14 ± 2.92	43.12 ± 2.78	0.91 ± 0.48	42.06 ± 2.68
Total	100.00	100.00	100.00	100	100

Table 5: Particle size analysis results for TB's prepared using different text references

Sample	Particle size			
	Mean (nm)	D10 (nm)	D50 (nm)	D90 (nm)
TB-A	55	24	50	84
TB-B	43	10	32	73
TB-C	49	12	42	81
TB-D	47	10	37	84
TB-E	53	19	45	88

Table 6 Pharmacokinetic Parameters of 5 Different TB Preparations

Parameters	TB-A	TB-B	TB-C	TB-D	TB-E
λ_z (1/h)	0.186	0.132	0.135	0.139	0.106
t1/2 (h)	3.72	5.23	5.11	4.97	6.49
Tmax(h)	48	48	48	48	48
Cmax (ppm)	82.21	58.90	67.30	74.75	48.69
AUC 0-t (ppm*h)	3007.17	2323.83	2996.19	2407.56	1790.54
AUC 0-inf_obs	3012.14	2340.27	3013.54	2424.25	1822.04
AUMC 0-inf_obs	102874.73	78152.64	98636	88902.32	1822.04
MRT 0-inf_obs (h)	34.15	33.39	32.73	36.67	34.73
Vz/Fobs ((mg)/	0.010	0.018	0.013	0.016	0.028

Figures

Figure 1: XRD pattern for TB prepared with different textual references

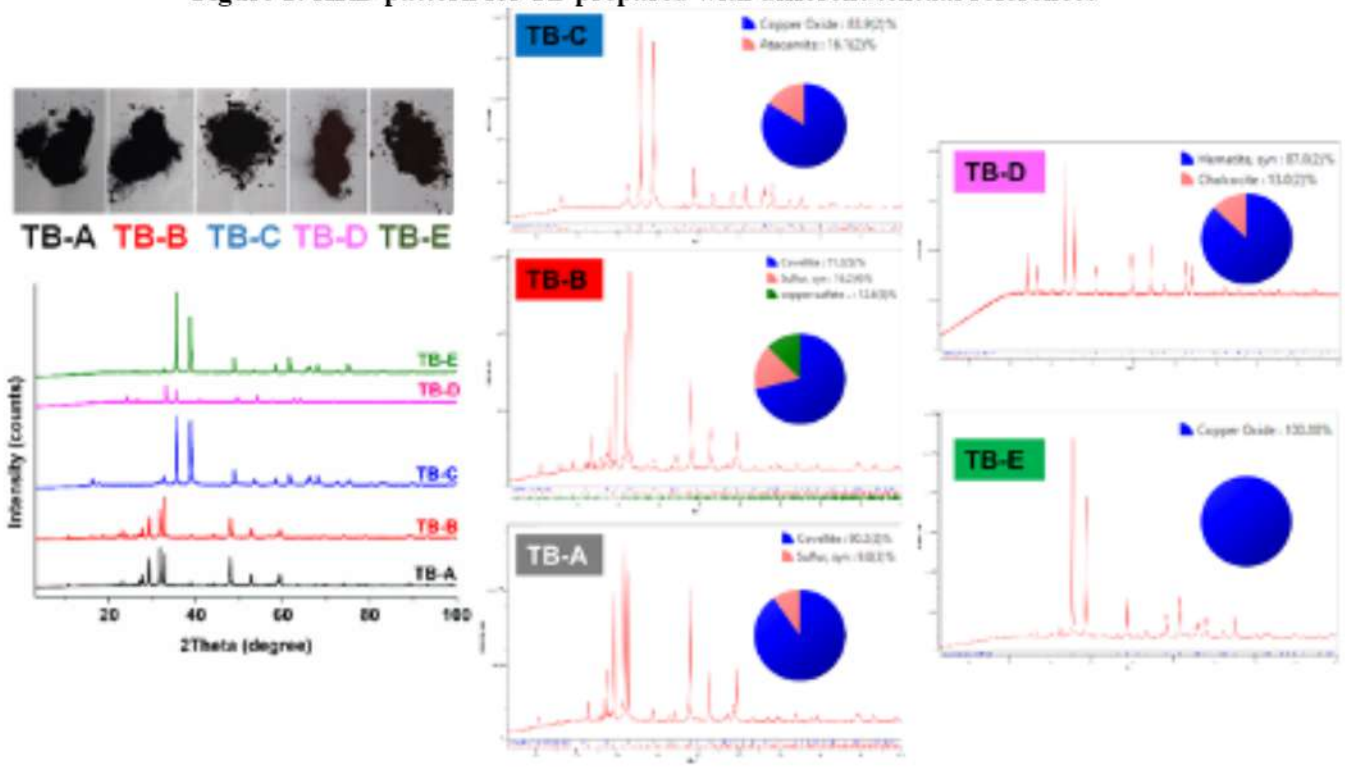


Figure 2: SEM (TB-A: A1, TB-B: B1, TB-C: C1, TB-D: D1, TB-E: E1) and EDAX (TB-A: A2, TB-B: B2, TB-C: C2, TB-D: D2, TB-E: E2) for TB prepared with different textual references

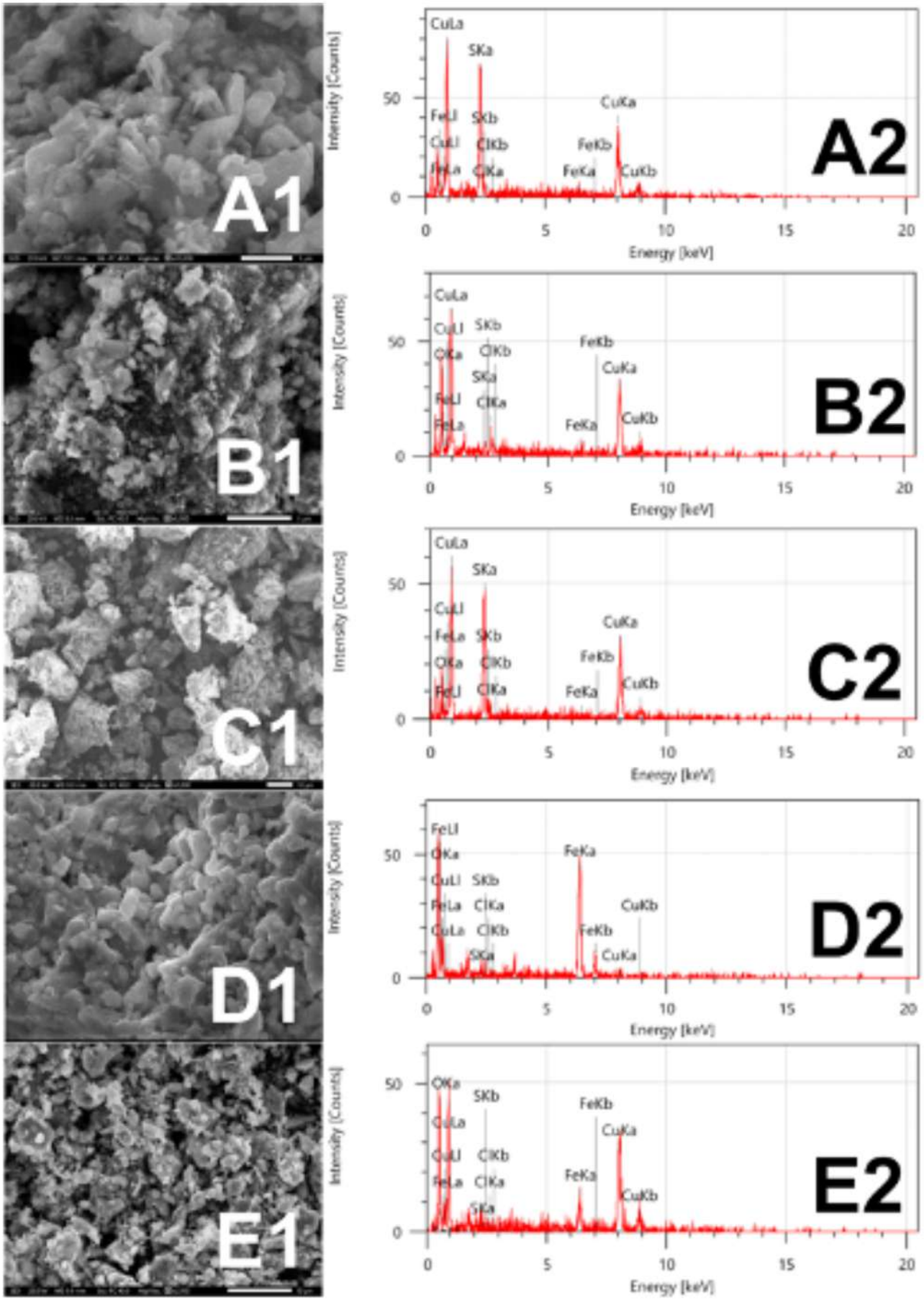


Figure 3: Particle size analysis (NTA) for TB prepared using different text references, A: TB-A, B: TB-B, C: TB-C, D: TB-D, E: TB-E (Inside each figure contains graph of relative intensity versus particle size and sample video frame)

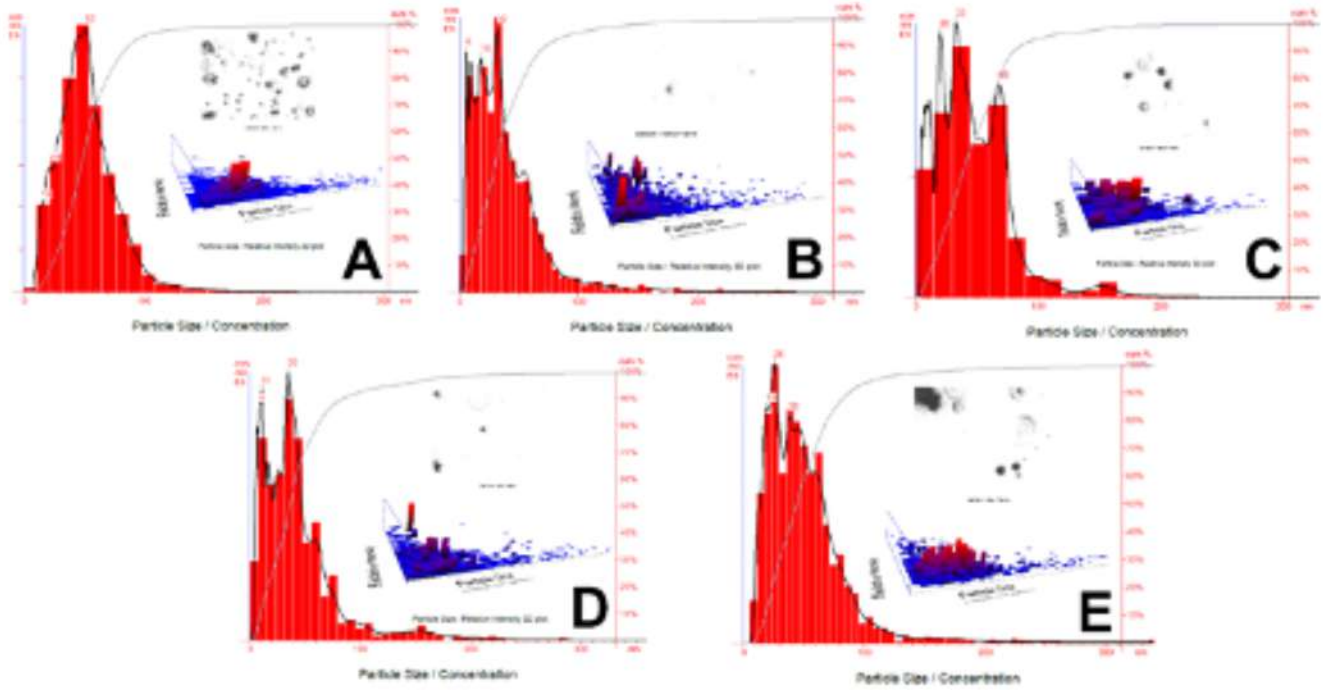
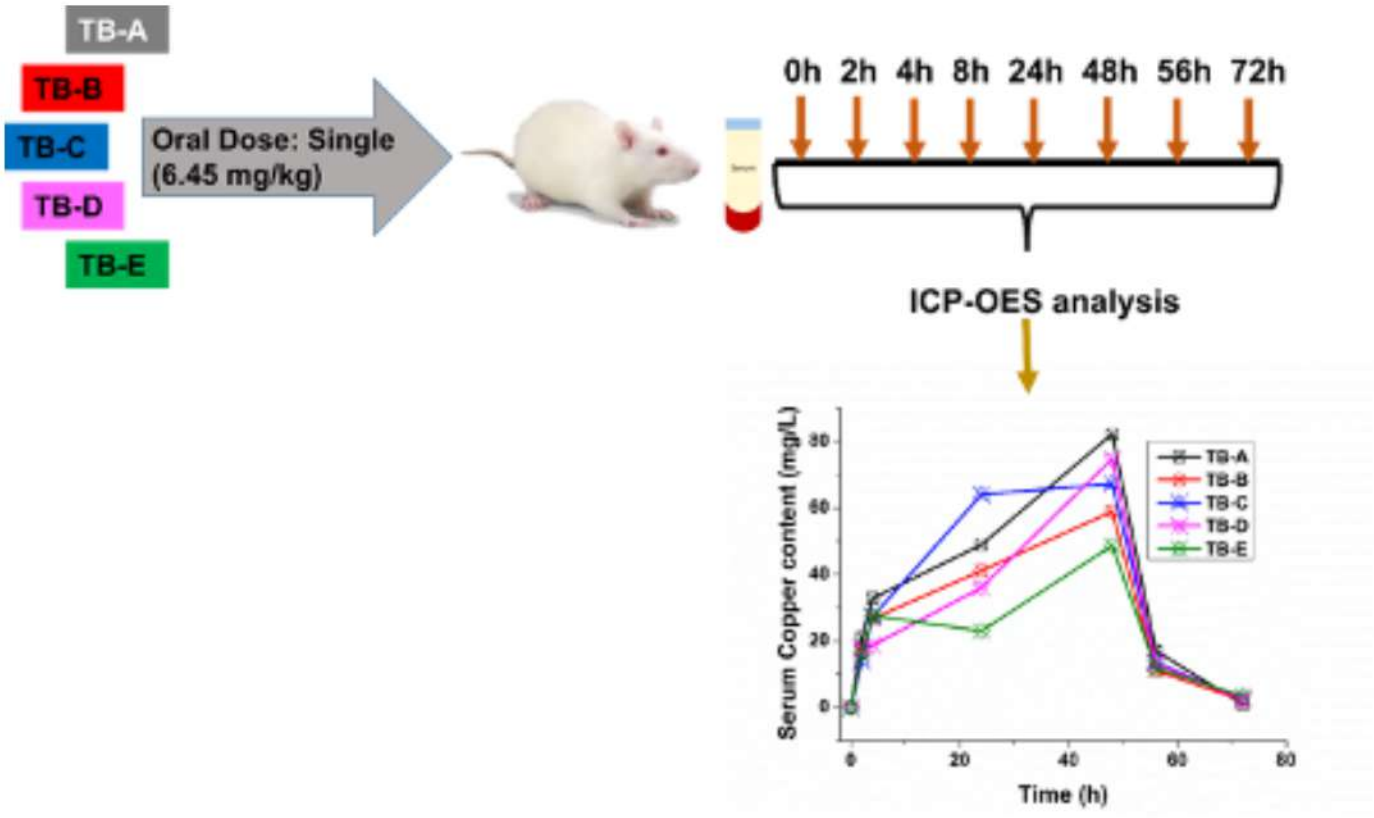


Figure 4: Graphical representation of Copper (Cu) content in serum
Bioavailability of Copper (Cu) in serum was established by measuring the time-dependent content of copper in the serum



Reference

1. Rasaratna sammuchaya, 1st Edition, Choukhamba Sanskrit Bhavan, Varanasi 1998.
2. WHO, Research guidelines for evaluating the safety and efficacy of herbal medicines. Malina, Philipines: WHO, Regional Office, Western Pacific Region: 1993.
3. Chaudhari S, Nariya M, Galib R, Prajapati P. Acute and subchronic toxicity of *Tamra bhasma* (incinerated copper) prepared with and without *amritikarna*. J Ayurveda and Integrative Medicine. 2016:7:23-29.
4. Pal D, Sahu CK, Haldar A. *Bhasma*: the ancient Indian nanomedicine. J Adv Pharm Tech Res.2014; 5(1):4–12.
5. Anonymous. The Ayurvedic Formulary of India. 2nd Ed. New Delhi: Gov of India. 2003; 236.
6. Jagtap C, Ashok B, Patgiri B, et al. Comparative antihyperlipidemic activity of *Tamra bhasma* (incinerated copper) prepared from *shodhita* (purified) and *ashodhita tamra* (raw copper). Indian J Nat Prod Resour. 2013; 4(2):205–211.
7. Pattanaik N, Singh AV, Pandey R, et al. Toxicology and free radicals scavenging property of Tamra bhasma. Indian J Clin Biochem. 2003; 18(2):181–189.
8. Honwad S, Bairy TS Ravishankar B. Hepatoprotective activity of Somanathi *Tamra bhasma* in paracetamol induced liver toxicity in albino-rats. J Phytopharmacol. 2015; 4(3):143–146.
9. Pandey BL, Goel RK, Das PK. A study of the effects of *Tamra bhasma*, an indigenous preparation of copper on experimental gastric ulcers & secretion. Indian J Exp Biol. 1983 May; 21(5):258-64. PMID: 6686832.
10. Goel RK, Maiti RN, Mukhopadhyaya K. Effect of *Tamra bhasma*, an Indian indigenous preparation of copper, on rat gastric mucosal resistance. Indian J Exp Biol. Aug 1994; 32(8):559-61. PMID: 7959937.
11. Sanyal AK, Pandey BL, Goel RK. The effect of a traditional preparation of copper, *Tamra bhasma*, on experimental ulcers and gastric secretion. J Ethnopharmacol. 1982 Jan; 5(1):79-89. doi: 10.1016/0378-8741(82)90023-x. PMID: 7054601.
12. Vahalia MK, Thakur KS, Nadkarni S, Sangle VD. Chronic toxicity study for *Tamra bhasma* (A generic ayurvedic mineral formulation) in laboratory animals. Rec Res Sci Tech. 2011:3(11):76-7.
13. Jagtap CY, Ashok BK, Patgiri BJ, Prajapati PK, Ravishankar B. Comparative anti-hyperlipidemic activity of *Tamra bhasma* (incinerated copper) prepared from *shodhita* (purified) and *ashodhita tamra* (raw copper). Ind J Nat Prod Res. 2013:4(2):205-211.
14. Jagtap CY, Ashok BK, Patgiri BJ, Prajapati PK, Ravishankar B. Acute and Sub-chronic Toxicity Study of *Tamra bhasma* (Incinerated Copper) prepared from *ashodhita* (Unpurified) and *shodhita* (Purified) *Tamra* in Rats. Indian J Pharm Sci. 2013:75(3):346-352.
15. Mohan, M., Saad, M., Tambe, S., Pande, S., Kulkarni, A., Bakare, S., & Mohite, A. Pre-clinical toxicity study of *Tamra bhasma* on albino wistar rats. International Research Journal of Pharmacy, 2018, *9*(1), 36–46. https://doi.org/10.7897/2230-8407.0916
16. Dabhikar GK, Kamble SB. Efficacy of *Tamra bhasma* on Lipid profile in Albino rats. Contemporary Res India. 2020:49-56.
17. Patil S, Kanase A, Varute AT. Effect of hepatoprotective ayurvedic drugs on lipases following CCl4 induced hepatic injury in rats. Indian J Exp Biol. 1989 Nov; 27(11):955-8. PMID: 2620934.
18. Singh R, Kumar S, Aman A, karim SM, Kumar S, Kar M. Study on physical properties of Ayurvedic nanocrystalline *Tamra bhasma* by employing modern scientific tools. J Ayu Integrative Med. 2019:10:88-93.
19. Wadekar MP, Rode CV, Bendale YN, Patil KR, Prabhune AA. Preparation and characterization of a copper based Indian traditional drug: *Tamra bhasma*. J Pharmaceutical biomedical Analysis. 2005:39:951-955.
20. Chaudhari S, Ruknuddin G, prajapati PK, Rao MM. Analytical specifications of *Tamra bhasma*. J Drug Res in Ayurvedic Sci. 2018:3(2):65-70.
21. Liu J et al. Chemical Compositions of Metals in *bhasmas* and Tibetan Zuotai Are a Major Determinant of their Therapeutic Effects and Toxicity. Evidence based Complementary Alt Med. 2019:1697804:1-13.

*****