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Process and Pharmacological Potentials of Kasisa bhasma, a Herbo-Mineral Formulation with Calcinated Ferrous Sulphate

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ABSTRACT

Objective: To process, standardize and assess the *Kasisa bhasma* formulation.

Methods: The *Kasisa bhasma* (AK1) was processed in strict accordance to Ayurvedic formulary of India. AK1 was characterized physicochemically by FT-IR, TGA, SEM, AFM, XRD and AAS techniques. Formulation was assessed for anti-anemic, hepatoprotective and toxicity potentials using albino rats. The commercially marketed *Kasisa bhsma* (AK2) formulation was also used for comparative study.

Results: The FT-IR spectra showing principal inorganic, hydrated metal salt or oxide peak was an indicative of bhasma. The XRD analysis of both formulations exhibited crystalline nature and nano-sized particles when determined using Scherrer formula. SEM showed well-defined plate like structures of ferric oxide in AK2 while AK1 showed spongy, relatively compact microcrystalline aggregates with loss of grain boundaries. AFM analysis confirmed the spherical morphology of AK1 and AK2 due to aggregation of nanocrystals of metallic oxides in the formulation. The presence of ferric oxide in nano-crystalline size was confirmed by TGA curves and quality by AAS study. The formulations AK1 and AK2 had exhibited anti-anemic and hepatoprotective potentials in albino rats. No toxicity was reported in histopathological study.

Conclusion: The schematic process of *Kasisa bhasma* imparts quality in the formulation and standardization protocols and may therefore be considered as a fingerprinting of *Kasisa bhasma* using sophisticated analytical techniques.

Keywords: Herbo-mineral, SEM, TGA, AAS, AFM, incineration, anemic, hepatotoxicity.

1. INTRODUCTION

Bhasma, a herbo-mineral Ayurvedic medicine gained its reputation as a very effective formulation against many chronic ailments compromising the aspects of nanotechnology and overcoming the limitations of conventional dosage forms[1]. The *bhasma* preparation essentially contains a metallic (toxic) element. But the metal in *bhasma* is processed into a non-toxic and, moreover, therapeutically enriched agent by a so-called traditional process, *bhasmikarana.* The *bhasmikarana* process involves two important steps. The first step is *shodhana* (roasting along herbal juices with continuous stirring) and the second step is *maarana* which involves *bhavana* (wet trituration) and *puta* system of heating. Thus, therapeutic value of metals in the *bhasma* relates to their ability to modulate them as nontoxic formulations[2]. *Bhasmas* are produced by repeated calcinations to obtain them either as ash or metallic nanoparticles with reduced particle size that may facilitate therapeutic moiety to absorb and assimilate into biological circulation. *Bhasmas* are usually administered along with milk,

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butter, honey and ghee that make them palatable, eliminating their harmful effects and enhancing their biocompatibility[3].

Kasisa bhasma, a calcinated iron preparation obtained from *Kasisa* or *Hirakosh* ($FeSO_4 7H_2O$-vitriol) is primarily used to treat anemia and also as immunomodulatory and dysmenorrhoea. Due to astringent property, it is being used in dysentery, diarrhea, haemorrhage, ulcers and vitiligo conditions[4,5,6]. The repeated calcinations of *Kasisa* impart the quality in resulting *bhasma* and facilitate particles to undergo submicron size. Best quality *bhasma* formulations are essentially required to treat the severe anemia and associated disorders. Calcinated formulations containing typically nano-size or submicron-size therapeutic moieties would alter certain immunogenic status by extended distribution phase and, thus, therapeutic outcomes of such *bhasma* formulations would evidently increase many folds[7]. Therapeutic efficiency of any formulation typically depends on the quality of starting materials and also on the technology adapted for particular formulation. Both these vary over space and finally reflect in the quality of formulation. The wrong and unskilled manufacturing practices pave way to the production of inferior quality products, which reduce efficacy and are devoid of safety. By following precise manufacturing practices with qualified starting materials and validated process, it is possible to minimize the variability in quality and also to prevent the adulteration in most potent heamatogenic formulation, *bhasma*. In this backdrop, the present research was focused on in-house process of *Kasisa bhasma* (AK1) with quality standards as per Ayurvedic formulary and on its pharmacological potentials. The commercial *Kasisa bhasma* (AK2) formulation was also used here for comparison.

2 Materials and Methods

2.1 Chemicalsand Animals:

Ferrous sulphate, a prime ingredient in the preparation of *Kasisa bhasma* was procured from SD Fine Chem Ltd., Mumbai, India. Commercial sample of *Kasisa bhasma and* other raw materials were obtained from the local market.

Male weanling Charles Foster strain albino rats weighing 90-100 g and Wistar strain albino rats of either sex (160-180 g) were separately maintained under standard laboratory conditions with 12-12 h light-dark cycle. The animals were fed with standard rat pellet (Lipton, India Ltd.) and *ad libitum*. The animals were acclimatized to the laboratory conditions for ten days before commencing the experiments. Experimental protocols were authenticated by the Institutional Animal Ethical Committee (Ref. No. IAEC/930/a/06/ CPCSEA).

2.2 Experimental

As per traditional *bhasmikarana* process, in the first step of *sodhana* process, ferrous sulphate (250 g) was mixed with appropriate volume (~2.5 L) of citrus fruit juice. The resulting mixture was triturated with *khalvam* to obtain a homogenous paste for a period of 3.5 h. The homogeneous mixture was further undertaken for the second step, *maarana* process. The mixture was placed in an earthen crucible covered with a lid. The junction was sealed with double folded, clay smeared cloth. The mixture was subjected to heat in an electrical muffle furnace at 650º C for first and second *puta* in span of 3.5 min. Almost 30 min time was consumed to reach high temperature whereas it took 4 h 30 min to fall to 30ºC. The incineration process was repeated for four more times to obtain the formulation of *Kasisa bhasma* (AK1)[8].

2.3 Physical standardization

The prepared *Kasisa bhasma,*AK1, was preliminary analyzed for floating property, fineness, metallic luster, colour, odour, taste, pH, total ash, acid insoluble ash, water soluble ash, loss on drying (LOD) and particle size.

2.4 Characterization studies

FT-IR analysis

The both AK1 and AK2 *bhasmas* were analyzed for changes in their chemical integrity by using Perkin-Elmer FTIR spectrophotometer (1600). Each spectrum of *bhasma* sample was collected from 16 single average

scans at a resolution of 4 cm^{-1} in the absorption region of 600-4000 cm^{-1}.

Thermogravimetric Analysis (TGA)

The obvious total weight change due to *maarana* (thermal) process in the *bhasma* formulations was assessed by NETZSCH Thermoanalyzer (STA–409). The changes in weight of AK1 and AK2 were recorded between temperatures 0 to 900º C.

Scanning Electron Microscopy (SEM)

The ultramicroscopic structures in AK1 and AK2 were examined using a scanning electron microscope (Hitachi S-3000N SEM). The formulations were fixed to a brass specimen club using double sided adhesive tape made electrically conductive by coating in a vacuum with platinum at 15 Ma.

Atomic Force Microscopy (AFM)

The both samples AK1 and AK2 were assessed for nanosized topography using Nanonics Multiview 1000 AFM head with E scanner (Nanonics Imaging Ltd., Israel). Scanning of images was performed by tapping AFM tips at 20 nm and oscillating the cantilever at its free resonance frequency (80 kHz). The exact position of the tip onto the sample was controlled using an inverted microscope (Olympus, Japan) mounted above the AFM. All measurements were performed at 20° C. The AFM images were captured, processed and analyzed with QUARTZ software, version 1.00 (Cavendish Instruments Ltd., UK).

XRD study

The crystallographic structure and crystalline phases of AK1 and AK2 formulations were examined by using an X'Pert Pro (Phillips) X-ray powder diffractometer. The samples diffraction patterns were studied by placing the *bhasma* samples in conventional cavity mounts and using Ni-filtered, CuKα radiation, a voltage of 40 kV and a current of 30 mA. The instrument was operated in the continuous scan mode over a 2θ range of 10 to 70º.

Atomic Absorption Spectrometric (AAS) analysis

The concentrations of iron and other trace elements present in AK1 and AK2 were assessed using Atomic Absorption Spectrometer (Perkin Elmer, USA). A 10 mg sample of *bhasma* was digested in 2 ml of aqua-regia and diluted appropriately to required volume. The concentration of iron was calculated by flame AAS.

2.5 Pharmacological standardization

2.5.1 Anti-anemic efficacy:

Male weanling Charles Foster strain albino rats were divided into two groups as: (i) anemic and (ii) non-anemic. Animals of anemic group were again subdivided into three groups according to three treatment schedule. Six animals were used in each.

Induction of anemia: The animals of anemic group were supplied standard agar gel diet[9]. The diet was prepared for every 6 days rationing at a time and ensured that there is no trace of iron by qualitative chemical analysis. Utmost care was taken to avoid additional iron contamination if any by supplying triple distilled water to animals under study. The non-anemic group animals were supplied agar gel diet in combination with a measured amount of ferrous sulfate and free access to tap water.

Phlebotomy: In anemic group animals, apart from agar gel feeding, anemia was induced by phlebotomy in each rat. In this method animal tail vein was punctured and 0.6 ml of blood was let out. Phlebotomy was carried out on 1, 4, 8 and 10th day after continuous feeding of the agar gel diet for 15 days[9].

Treatment schedule: Animals of three anemic groups were treated orally withAK1 (0.02 mg/kg) *bhasma* formulation in 2% gum acacia suspension, commercial AK2 (0.02 mg/kg) *bhasma* formulation in 2% gum acacia suspension and 2% gum acacia suspension, respectively. The animals received treatment orally through feeding canula daily from 15th day after starting of agar gel diet and up to 60th day. Non-anemic animals were not treated with formulations and were considered as untreated control group. Kaur N (1994) method was adopted to calculate the treatment doses in the study[10].

Body weight: The change in body weight (in g) of

each animal was recorded at 15-day intervals.

Estimation of hemoglobin: Hemoglobin level of each animal was estimated by cyanmethemoglobin method. Animal blood was collected on 0, 4, 8, 10, 12, 30, 45, 60 and 75 days. The hemoglobin regeneration efficiency (HRE) was also calculated using formula[11].

$$\text{HRE}\,(\%) = \frac{(\text{mg final Hb Fe - mg initial Hb Fe})}{\text{mg Fe consumed}} \text{ x } 100$$

Estimation of serum ferritin: The serum ferritin level of each animal was estimated by ELISA reader[12]. The ferritin assessment was carried out between 15 and 75 days of treatment period.

Estimation of serum iron and total iron binding capacity (TIBC): Serum iron and TIBC of each animal were estimated spectrophotometrically from the collected serum at 562 nm[13].

2.5.2 Hepatoprotective efficacy

Carbon tetrachloride (CCl_4)-induced hepatotoxicity in rats[14]

Thirty albino rats of Wistar strain were randomly divided into 5 groups, each of six animals. Group 1 animals served as control and animals in group 2 served as untreated group. Both I and II group animals received 3 doses of 5% acacia solution (1 ml/kg, p.o.) at 12 hr intervals. Group II animals received CCl_4 (1.25 ml/kg, i.p.) along with liquid paraffin (1:1) half-an hour after 1st dose of vehicle. Animals in groups III to III were administered AK1, AK2 and silymarin (reference standard) a oral dose of 0.02, 0.02 and 100 mg/kg, respectively, by gastric intubation with a soft rubber catheter three times, at 0, 12 and 24 hr. CCl_4 was injected intraperitonially 30 min after the 1st dose. After 36 h of CCl_4 treatment, blood was collected from all rats and serum was separated by centrifugation. Various biochemical parameters were analyzed in serum by auto analyzer (Mispa Excel) such as SGOT, SGPT, alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (γ-GT), total proteins (TP) and total bilirubin.

2.5.3 Toxicology and histopathological studies

The acute toxicity of *Kasisa bhasma* was studied by following OECD-423 guidelines[15]. In this toxicity study, nine female albino rats were randomly divided into 3 groups (control, AK1 and AK2) of each three. Overnight-fasted animals of AK1 and AK2 were administered single oral dose of 0.02 mg/kg. The animals were monitored carefully after dosing during first 30 min. Food was withheld for further 3-4 h after administration of AK1, AK2. Treated animals were closely observed for first 4 hr and daily thereafter, for a total of 14 days. As there was no lethality observed at this dose level, the procedure was repeated further dose of 2 mg/kg. The animals were observed for toxicity signs such as salivation, diarrhea, lethargy, sleep and coma and also gross behavioural changes in skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic, central nervous system and motor activity. The animals were sacrificed and their organs were carefully isolated. The tissues of stomach, kidney and liver were fixed in 10 % formalin embedded in paraffin wax. Histological sections were cut at 4-5 µm thickness and stained with hematoxylin-eosin. The photomicrographs of tissues were captured at various magnifications using Zeiss optical microscope (2000-C), with an attached trinocular camera.

2.6 Statistical analysis

The significance of differences among the groups was assessed using one-way ANOVA followed by Tukey-Kramer test for multiple columns comparison. The value $p<0.05$ was considered as statistically significant.

3 Results

3.1 Physical standardization

Kasisa bhasma, AK1 formulation was produced in strict accordance to the traditional process *bhsamikarana*. Produced AK1 formulation appeared typically in brick red color, was tasteless and exerted characteristic odour at pH7.4. The AK1 was found floating when sprinkled on the surface of water and entered into the lines of the fingers when rubbed between the fingers thus passing the fineness test. The loss of metallic luster was confirmed

when exposed to sunlight as there was no brilliance and shine of metal. The same characteristics were observed in commercial formulation of AK2 as well. The physical properties such as total ash, acid insoluble ash, water soluble ash and %LOD are shown in Table 1. The particle size of AK1 and AK2 was analyzed with particle size analyzer.

3.2 Characterization studies

The FTIR spectra of AK1 and AK2 are shown in Figure 1. The spectra were evident with the principal peaks correspond to inorganic metal (Fe_2O_3), hydrated metal salt or oxide (FeO or Fe_3O_4). The peaks related to any organic molecule or corresponding bonds were absent in spectra.

The Figure 2 of TGA curves showed complete decomposition of AK1 and AK2 at 1232 and 1279 ºC that indicated the presence of ferric oxide in formulations which was converted upon incineration of ferrous sulphate in the presence of atmospheric air.

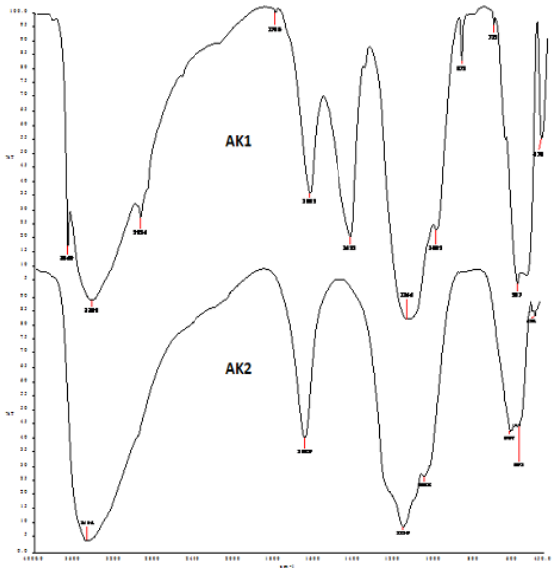

Figure 1: FTIR spectra of AK1 and AK2

Table 1. Physical parameters of AK1 and AK2

Parameter	AK1 (%)	AK2 (%)
Total ash	100	100
Acid insoluble ash	68.4	50.35
Water soluble ash	4.28	3.19
Loss on drying	1.0	2.0

SEM images of AK1 and AK2 showed difference in size and agglomeration of the particles (Figure 3). Agglomeration of the particles is due to the repetition of calcinations cycles for the preparation. In AK2, ferric oxide showed well-defined plate-like structures whereas AK1showed spongy, relatively compact microcrystalline aggregates with loss of grain boundaries.

AFM analysis confirmed that both formulations, AK1 and AK2 possessed spherical morphology with mean particle size of 50 and 100 nm, respectively, as shown in Figure 4.

The XRD pattern of both AK1 and AK2 is shown in Figure 5. The diffraction angles of AK1 are at 34.8, 36.4, 49.7, 55.3, 63.9 and 65.1º whereas diffraction angles of

AK2 are found at 11.2, 15.6, 16.1, 34.5, 36.9, 42.1, 50.9, 56.4, 63.1 and 65.6º. Comparative analysis of XRD results between AK1 and AK2 shows that the major reflection peaks of both samples are at identical positions. The high intensity of peaks in the XRD suggests that the drug is present in crystalline state. The size of crystallites in *Kasisa bhasma* was calculated from the XRD pattern using the Scherrer formula and found to be in the range of 53 - 57 nm. The results of AAS study revealed that 65.54% and 85.91% of elemental iron are present in AK1 and AK2 formulations, respectively.

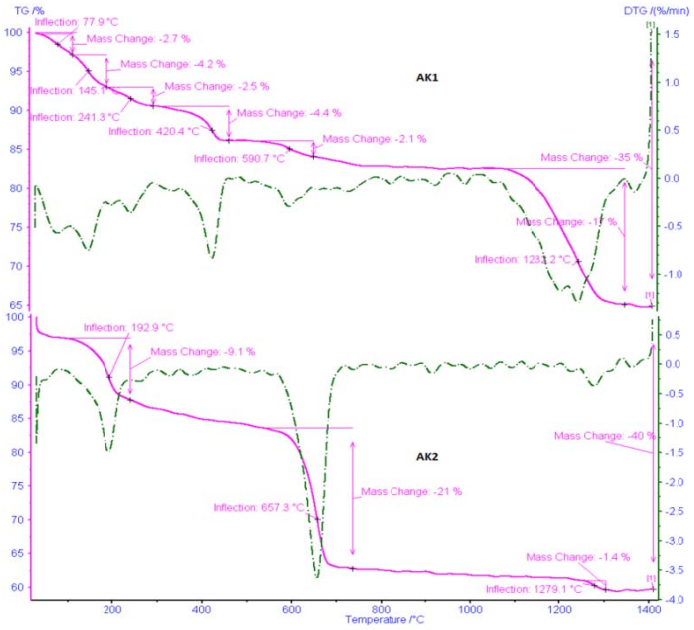

Figure 2: TGA curves of AK1 and AK2

Table 2. Mean hemoglobin level (%) of rats in different treatment groups

Day	Non-anemic group	Anemic group		
		Control	AK1	AK2
0	11.75 ± 1.32	11.37 ± 1.67	11.63 ± 1.02	11.02 ± 1.32*
4	11.75 ± 1.27	11.06 ± 0.73	10.60 ± 1.23	10.03 ± 0.90*
8	11.76 ± 1.36	10.53 ± 0.96	08.02 ± 0.69	08.89 ± 1.30*
10	12.57 ± 1.76**	09.67 ± 0.90	07.93 ± 0.21	07.90 ± 0.22*
12	12.79 ± 1.43**	07.34 ± 1.01	06.63 ± 0.25	06.53 ± 1.20*
30	13.43 ± 1.17**	07.37 ± 1.30	08.62 ± 0.12*	08.53 ± 0.72**
45	13.77 ± 1.01**	07.73 ± 1.01	08.90 ± 0.11*	08.94 ± 0.71**
60	14.43 ± 0.65**	08.01 ± 0.02	12.32 ± 1.30*	11.24 ± 1.90**
75	14.54 ± 0.75**	08.12 ± 0.13	13.01 ± 0.12*	13.70 ± 1.17**

n=6 in each treatment group
*p<0.05; **p<0.001 as compared to control
All values are expressed as mean ± S.D.
Control group treated with 2% gum acacia as vehicle

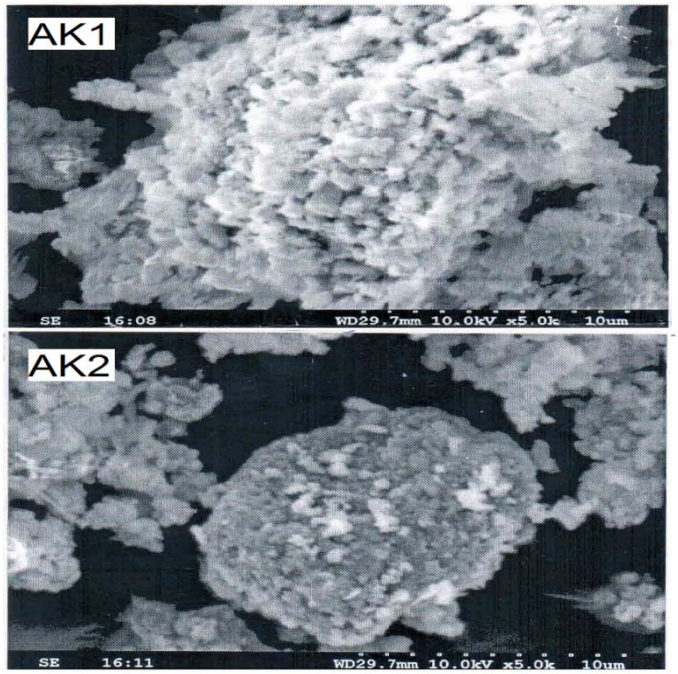

Figure 3: SEM of AK1 and AK2

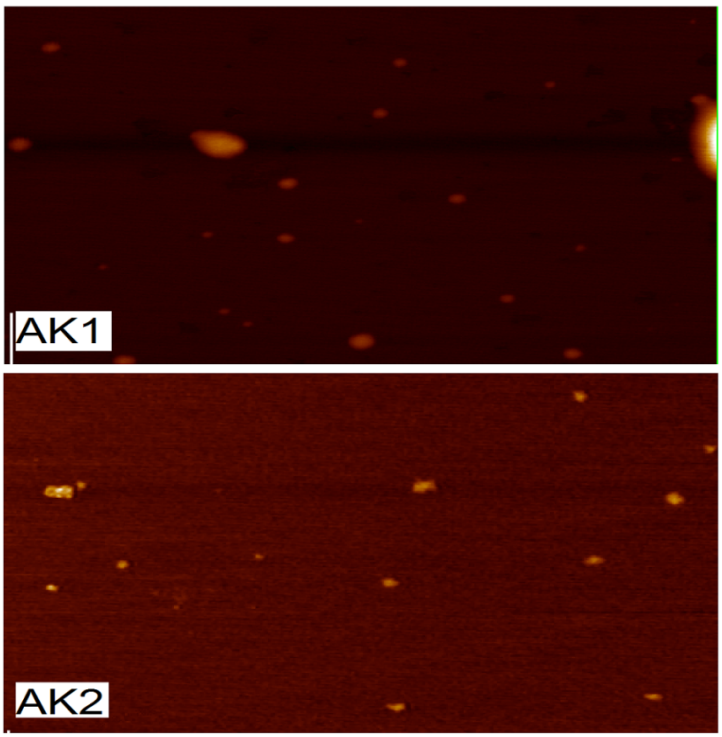

Figure 4: AFM photograph of AK1 and AK2

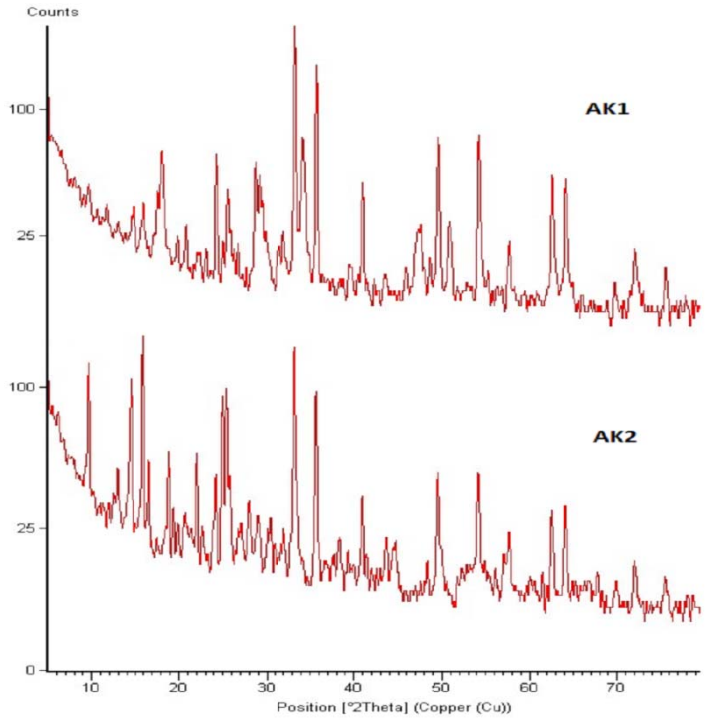

Figure 5: XRD patterns of AK1 and AK2

3.3 Anti-anemic Activity

Increased hemoglobin levels were noticed at 75th day in anemic group animals as shown in Table 2 indicating anti-anemic activity of AK1 and AK2. The animals of anemic groups which received treatment with *bhasma* formulations had increase in body weights (Table 3).

The present study reveals that, when treated with AK1 and AK2, a significant decrease of TIBC level was observed (Table 4). The commercial *bhasma* formulation AK2 seems to be slightly better in this regards than formulated bhasma AK1. Another important observation was the increase in the serum iron level of AK1 and AK2-treated groups.

3.4 Hepatotoxicity study

The effect of both *Kasisa bhasma* on CCl_4-induced hepatotoxicity is presented in Table 5. The animals treated only with CCl_4 exhibited a significant increase ($p<0.001$) in SGOT, SGPT, ALP, γ-GT and total bilirubin levels as well as decrease in TP levels when compared to the control group after 36 h. The *bhasmas* at tested doses produced a significant reduction ($P<0.001$) in the CCl_4-induced elevated levels of SGOT, SGPT, ALP, γ-GT and total bilirubin as well as increase in the levels of TP when compared to the untreated group (that received CCl_4 alone) after 36 h.

3.5 Toxicological study

The AK1 and AK2 showed no toxicity in rats, and their body weights remained constant even after treatment with AK1 and AK2. The consistent and conventional structures of cells of stomach and kidney were maintained as observed in histopathological studies (Figure 6) indicating no renal and gastrointestinal toxicity.

Liver tissues of control group animals showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein. Disarrangement of normal hepatic cells with centrinobular necrosis, vacuolization of cytoplasm and fatty degeneration were observed in CCl_4-

intoxicated rats. The liver sections of the group 3 and 4 rats treated with AK1 and AK2 at the oral dose of 0.02 mg/kg showed signs of protection as it was evident by the moderate accumulation of fatty lobules, absence of necrosis and vacuoles (Figure7). Almost similar signs of protection were observed with silymarin treatment.

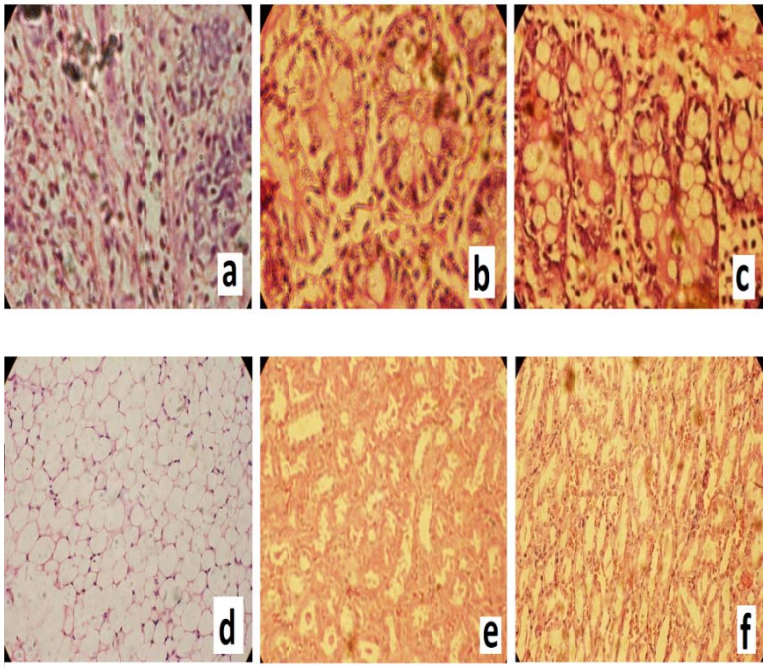

Figure 6: Histopathology of Stomach treated with (a) Control; (b) AK1; (c) AK2 and Kidney treated with (d) Control; (e) AK1; (f) AK2

Table 3. Mean body weight of rats in different treatment groups

Day	Non-anemic group	Anemic group		
		Control	AK1	AK2
0	91 ± 2.06	95 ± 1.52	94 ± 2.45	96 ± 1.44
15	122 ± 5.75**	106 ± 2.97	107 ± 5.96*	108 ± 5.55*
45	185 ± 4.63**	143 ± 8.31	181 ± 7.05**	177 ± 3.72**
75	217 ± 4.28**	169 ± 1.22	215 ± 4.05**	217 ± 7.26**

n=6 in each treatment group
**p*<0.05, ***p*<0.001 as compared to control
All values are expressed as mean body weight (g) ± S.D
Control group treated with 2% gum acacia as vehicle

Table 4. Mean serum ferritin, TIBC and serum iron at 15th day and 75th day of different treatment groups on albino rats

Day	Non-anemic group	Anemic group		
		Control	AK1	AK2
		Mean serum ferritin (mg/L)		
15	13.32±1.10	72.76±2.72	79.53±3.21	84.30±3.90
75	111.70±1.82*	58.42±2.32	84.90±2.70*	97.63±2.32*
		TIBC (µmol/L)		
15	86.37±2.32	102.45±2.83	97.08±4.73	92.02±3.52
75	87.32±2.93*	107.49±2.53	95.11±3.07*	90.70±6.03*
		Serum iron (µmol/L)		
15	18.12±1.62	9.73±1.32	10.79±1.30	11.73±1.72*
75	18.10±1.03*	9.03±2.33	16.77±1.27*	16.93±2.03*

n=6 in each treatment group
*p<0.001 as compared to control
All values are expressed as mean ± S.D.
Control group treated with 2% gum acacia as vehicle

Table 5. Effect of *Kasisa bhasma* on hepatic enzymes and serum bilirubin in rats after 36 h of CCl_4 treatment

Biochemical parameters	Group-I (Control)	Group-II (CCl_4:1 mL/kg)	Group-III (AK1: 0.02 mg/kg)	Group-IV (AK2: 0.02 mg/kg)	Group-V (Silymarin:100 mg/kg)
SGOT (U/L)	30.45±0.03***	61.59±0.17	45.12±0.01***	40.77±0.12***	33.06±0.01***
SGPT (U/L)	18.37±0.05***	38.53±0.19	34.36±0.04***	27.98±0.03***	22.85±0.06***
ALP (U/L)	188.6±0.04***	413.57±0.65	277.7±0.03***	243.8±0.07***	200.6±0.08***
γ-GT (IU/L)	50.40±0.03***	99.53±0.21	60.06±0.03***	57.85±0.03***	51.54±0.01***
TP (gm/dl)	7.45±0.04***	3.01±0.20	4.54±0.01***	6.6±0.07***	7.53±0.02***
Total bilurubin (mg/dl)	0.69±0.01***	3.53±0.11	1.73±0.02***	0.93±0.01***	0.72±0.02***

Values are expressed as mean±SEM of 6 rats in each group
*** p<0.001 as compared to CCl_4treated group
SGOT= Serum glutamate oxaloacetate transaminase; SGPT = Serum glutamate pyruvate transaminase;
ALP= Alkaline phosphatase; γ-GT = Gamma glutamyltranspeptidase; TP= Total protein
Control group treated with 2% gum acacia as vehicle

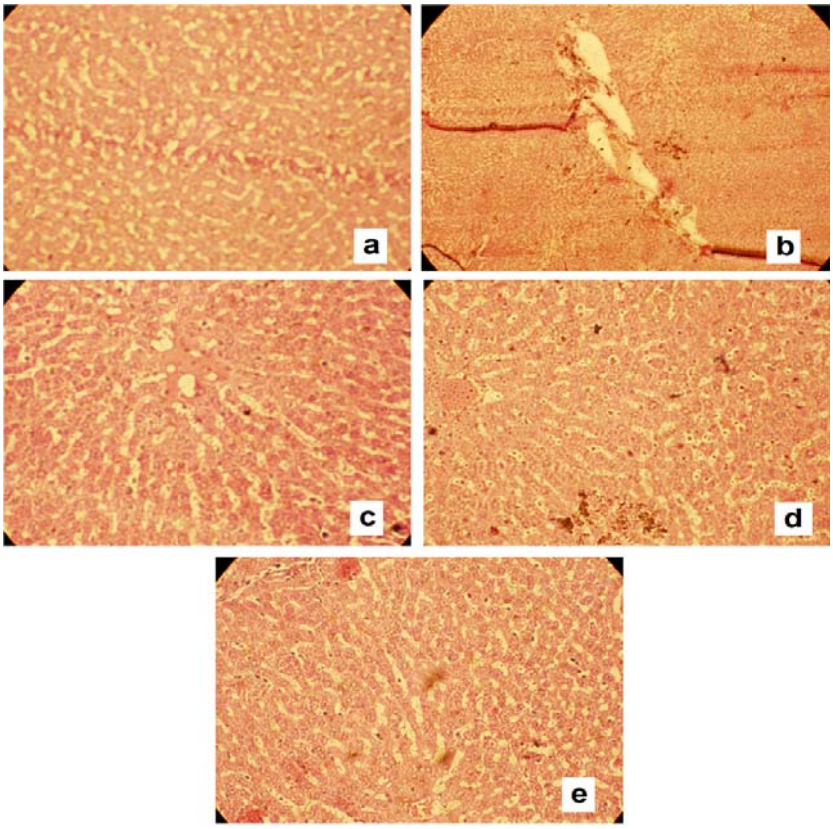

Figure 7: Histopathology of liver treated with (a)Control, (b) CCl_4, (c) AK1, (d) AK2 and (e) Silymarin

4 Discussion

Increase in interest on usage of Ayurvedic medicines in and around India is prevailed since recently. On the other hand, more new Ayurvedic products are coming into the market with or without validation. The *Kasisa bhasma* is among them and is known forages due to its high anti-anemic potential according to Indian system of medicine. Since formulation is enriched with iron content, it is apt to use in the treatments of iron deficiency and its associated cases. Therefore it is essential to formulate quality built-in *Kasisa bhasmas* with validated protocols in order to get high therapeutic outcomes in the treatment of anemia without toxicity.

As per the Ayurvedic formulary of India, repeated incineration of ferrous sulphate with schematic *putas* keep the powdered *bhasma* formulation without metallic luster (absence of elemental metal) and also facilitates possession of crystalline architect as noticed in X-ray diffractograms. A spherical morphology was displayed in SEM image which is due to the reversible aggregation of crystalline metallic oxides, and, as per AFM analysis (Figure 4), *bhasma* particles were distributed evenly in submicron/nano size range and displayed with bell-shaped histogram[16]. The presence of nano-sized ferric oxide as a metallic oxide was confirmed in TGA curves. Due to extreme high temperature treatment in the formulation and development of *bhasma*, the *bhasma* becomes more patient-compliant and contamination-free formulation. The process also ensured that there were no traces of organic contamination in *bhasma* formulation

which was evidently confirmed by the FT-IR spectra free from organic matter and external organic contamination[17].

Loss of body weight is one of the clinical manifestations in iron deficiency anemia. Increase in the body weight in the animals of treated (anemic) groups could be due to regaining of haemoglobin levels to an extent. Re-establishing haemoglobin occurred due to *Kasisa bhasma* treatment which has high iron content as noticed from AAS study and, moreover, due to nanocrystalline iron oxide state. Thus, in turn facilitated the increase in serum ferritin levels and are indicative of the total available iron. The results of our study indicated the *bhasmas* has some role in dissociating the bound iron from serum protein resulting in an increase in iron level in the serum. It is well recognized that in case of iron-deficiency anemia the serum proteins bind the free iron, and the binding capacity (TIBC) increases with the progression of the disease[8]. The *bhasma* formulations have outplayed in dissociation of serum protein-iron complex, thereby in increase in serum iron levels and thus *bhasma* will help in curing the iron deficiency anemia[2,18] .

Kasisa bhasma formulations re-established all biochemical parameters which were altered due to hepatocellular damage caused by CCl_4 toxicity. In group III to V animals, reduction of the levels of hepatic enzymes (SGOT, SGPT and ALP) and total bilirubin to the base levels indicates the re-generation process. This could be due to stability of biliary function and also due to the stabilization of endoplasmic reticulum that could stimulate protein synthesis[19,20]. Histopathological study showed that AK1 and AK2 have good potentials against hepatotoxicity in Wistar rats and the hepatoprotective activity of *bhasma* is on par with standard drug candidate sylimarin. Additionally, AK1 and AK2 are proven to be safe without leaving any toxicity signs in their gross behavior as well as histopathology of kidney and stomach.

5 Conclusion

The herbo-mineral formulation, *Kasisa bhasma* that contains iron as the active ingredient was processed successfully in strict accordance to Ayurvedic formulary of India and evaluated. The nano-size particles-containing *bhasma* formulations were analyzed by modern analytical techniques in order to specify the criterion for the final product conforming to all the traditional Ayurvedic parameters. The results of the present study revealed that *Kasisa bhasma* is an efficient iron preparation for the management of iron deficiency anemia. Overall, in-house prepared *Kasisa bhasma* formulation is very effective against severe anemia and iron deficiency-associated diseases with scientific evidence. Thus, the adapted process in the preparation of AK1, can be exploited for further establishment of validated protocols for Ayurvedic medicines and also as a basis for clinical studies.

REFERENCES

(1) Wadekar, M.P., Rode, C,V., Bendale, Y.N., Patil, K.R., Gaikwad, A.B. and Prabhune, A.A. Effect of calcination cycles on the preparation of tin oxide based traditional drug: Studies on its formation and characterization. *J. Pharma. Biomed. Anal*., 2006; 41:1473-1478.

(2) Jagtap, C.Y., Ashok, B.K., Patgiri, B.J., Prajapati, P.K. and Ravishankar, B. Acute and subchronic 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.

(3) Tripathi, Y.B. and Singh, V.P. Role of *Tamra bhasma*, an Ayurvedic preparation, in the management of lipid peroxidation in liver of albino rats. *Indian J. Exp. Biol*,. 1996; 34 (1):66-70.

(4) Arvind, K., Sharma, S. and Gaurav S. A rapid and simple scheme for standardization of poly herbal drugs. *Int. J.Green Pharm.*,2010;4:134-135.

(5) Dhirajsingh, R., Tekale, G.S. and Patgiri, B.J. Comparative study on *Kasisa bhasma* prepared by two

different methods. *Anc, Sci, Life.*, 2011; 31 (2): 62-72.

(6) Neogy, G.N., Sigh, R.R.K., Jamadagni, S.B., Mitra. A., Upadhyay, S.N. and Hazra, A. Toxicological evaluation of *Kasisa bhasma*, an Ayurvedic organo-metallic preparation. *Int. J. Res. Ayurveda Pharm.*, 2012; 3 (3): 381-386.

(7) Rinku, D.U. and Kishore, M.P. Jasada bhasma, a Zinc-based Ayurvedic preparation: contemporary evidence of antidiabetic activity inspires development of a nanomedicine. *Evidence-Based Complement. Alter. Med.,* 2015: 9 pages.

(8) Pandit, S., Biswas, T.K., Debnath, P.K., Saha, A.V., Chowdhury, U., Shaw, B.P., Sen, S. and Mukharjee, B. Chemical and pharmacological evaluation of different Ayurvedic preparations of iron. *J. Ethnopharmacol*., 1999; 65(2):149-156.

(9) Shivakumar, B., Madhavan, K. and Prasad Rao. Pharmacokinetics of norethisterone and levonorgestrel in experimental iron deficiency anaemia in rabbits. *Contraception.* 1985;32 (5):471-481.

(10) Kaur, N. and Kawatra, B.L. Effect of cooking methods and maturity of mustard leaves of bioavailability of iron. *J. Food Sci. Tech.*, 1994;31:285-288.

(11) Freireich, E.J., Gehan, E.A., Rall, D.P., Schmidt, L.H. and Skipper, H.E. Quantitative comparison of toxicity of anticancer agents in mouse, rat, dog, monkey and man. *Canc. Chemo. Rep*., 1966;50:219–245.

(12) Varley, H., Gowenlock, A.H. and Bell, M. Practical Clinical Biochemistry. London, 1980.

(13) Mahoney, A.W. and Hendricks,D.G. Nutritional Bioavailability of Iron. Washington: *American Chemical Society*; 1982.

(14) Acute Oral Toxicity – Acute Toxic Class Method, guideline 423, *OECD guideline for testing of chemicals*. (Organization for Economic Co-operation and Development, Paris) 2001.

(15) Suresh, S.V. and Misra, S.H. Hepatoprotective effect of *Pelgularia daemia* (Forsk.) ethanol extract and its fraction. *Indian J. Exp. Biol.,* 2008; 46: 447-452.

(16) Mishra, A., Mishra, A.K., Tiwari, O.P. and Jha, S. In-house preparation and characterization of an Ayurvedic bhasma: Praval bhasma. *J. Integr. Med.,* 2104; 12 (1): 52-58.

(17) Swapnil, Y.C., Dhirajsingh, S.R., Galib, R. and Pradeep, K.P. Fourier transform infrared analysis of *Tamra Bhasma* at different levels: A preliminary study. *Ayu*., 2015; 36 (1)-77-82.

(18) Bennet, P.N. and Brown, M.J. Clinical pharmacology. 2006; 9thed. New Delhi, India.

(19) Joshi, N., Sharma, K., Peter, H. and Dash, M.K. Standardization and quality control parameters for *Mukta bhasma* (calcined pearl). *Anc. Sci. Life*. 2015; 35 (1) 42-51.

(20) Garg, M., Das, S. and Singh, G. Comparative physicochemical evaluation of a marketed herbomineral formulation: *naga bhasma*. *Indian J. Pharm. Sci.*, 2012; 74 (6): 535-40.

وضع الطريقة المعيارية وفحص الإمكانيات الدوائية لنبتة كاسيسا فاسما مصوغ، نباتي–معدني مع كلسات كبريتات الحديدوز

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ملخص

الهدف: وضع الطريقة المعيارية وفحص الامكانيات الدوائية لنبتة كاسيسا فاسما.

الطرق: تم معالجة مصوغ كاسيسا فاسما (AK1) وفقا لكتاب الوصفات الايورفيدا في الهند. وقد تم فحص مواصفات الكيمياء النباتية ل AK1 التي عن طريق التقنيات التالية: AAS FT-IR, TGA, SEM, AFM, XRD. تم تقييم مصوغ لمكافحة فقر الدم، وحماية الكبد وإمكانية السمية باستخدام الجرذان. وقد استخدمت تركيبة (AK2) المسوقة تجاريا أيضا للدراسة والمقارنة.

النتائج: أطياف FT-IR أظهرت الأملاح غير العضوية، لمعدن المجود في النبتة أو القمة الخاصة بألكسيد الرطب والتي أشرت بشكل أساسي إلى وجود كاسيسا فاسما. اظهر التحليل باستخدام حيود الأشعة السينية لكل الصيغ طبيعة بلورية وجزيئات من حجم النانو. عند استخدام SEM مع معادلة شيرر ظهرت لوحة واضحة المعالم من هياكل أكسيد الحديديك في AK2 في حين ظهرت AK1 على شكل اسفنجي مدمج من وحدات البلور بحجم الميكرو مع فقدان لحدود الحبيبات. أكد تحليل AFM مورفولوجيا كروية من AK1 وAK2 بسبب تراكم البلورات النانوية من الأكاسيد المعدنية في الصياغة. وأكد وجود أكسيد الحديديك في حجم النانو البلورية التي أكدتها منحنيات TGA والجودة من خلال طريقة ASS. الصياغات AK1 وAK2 أظهرت إمكانات مكافحة فقر الدم وحماية الكبد في الفئران البيضاء. ولم ترد تقارير عن السمية في الدراسة التشريحية المرضية.

الخلاصة: هذه الدراسة تقدم طريقة لتحضير كاسيسا فاسما مما يضفي جودة في صياغة وتوحيد البروتوكولات وبالتالي يمكن اعتبار هذه الطريقة كطريقة معيارية لبصمات لكاسيسا فاسما باستخدام التقنيات التحليلية المتطورة.

الكلمات الدالة: نباتي–معدني، فقر الدم، تسمم الكبد.

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