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Evaluation of Structural, Chemical Characterisation and Safety Studies of *Samagandhak Kajjali*, an Indian Traditional Ayurvedic Drug

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ABSTRACT

Ayurveda, literally means "science of life" and it is practised over 5000 years back onwards. In *Ayurveda*, several compounds of Mercury (*Parada*) and Sulphur (*Gandhaka*) are extensively used in *Ayurvedic* therapeutics for a wide variety of ailments and conditions. *Kajjali* is one such compound which is the most predominant amongst them. *Kajjali*, an Indian traditional drug has been used in the treatment of various disorders. In our study, processing and chemical characterization of this drug using various techniques, viz. *X-ray diffraction* (XRD), *Scanning electron microscopy* (SEM), *X-ray photoelectron spectroscopy* (XPS), Particle size analyzer, *Thermo-gravimetry analysis* (TGA), and *Energy dispersive X-ray fluorescence* (EDXRF) have been reported. In the perspective of safety concerns *In vitro* bovine shrimp assay and Osmotic fragility test also have been performed. XRD pattern of preparation delineats its cubic and hexagonal form with 2θ position at 23.08, 26.42,27.76 28.80,30.46, 31.25, 43.78, 51.86, and 54.30 with d-spacing of 3.83, 3.37, 3.21, 3.09, 2.93, 2.86, 2.06, 1.76 and 1.68 Aº respectively. SEM photomicrograph of *Samagandhak Kajjali* particles shows the appearance of particles of 10 um and less than 5 um size particles i.e. Upto 0.237 um in size. Drug contains Mercury in the Mercury Sulphide (HgS) form with free Sulphur and associated with organic contents, whereas EDAX showed the presence of Hg (49.4 %), S (48.70 %), Zn (12.39 ppm), P (0.67%) and Selenium (0.04 ppm) in the final preparation of *Kajjali*. There is no significant ($p< 0.05$) difference in the *In vitro* toxicity and Osmotic fragility of control group with treated ones. These findings help in understanding the therapeutic value, safety aspect and standardization of Ayurvedic drug- *Kajjali*. Though the metallic Mercury is known to be toxic to the biological system, no compelling evidence has been put forth to suggest any toxic effects of *Kajjali*. By observing the results of structural and chemical characterisation of the study, clearly delineats in crystal view manner of its safety concern.

Keywords: Chemical Characterisation, *Kajjali*, Structural analysis

1. Introduction

Ayurveda – literally meaning science of longetivity – puts greater emphasis on prevention of disease rather than cure, by maintaining positive health through holistic approach [1]. It mainly aims to improve health through the use of effective rejuvenators as well as thorough proper diet. For this purpose *Ayurveda* has chosen all possible materials from three natural resources of– herbal, metal/mineral and animal origin. Subsequently, a branch dealing with use of medicines predominantly of metal/mineral origin, emerged by the name '*Rasashastra*' (*Rasa*= Mercury, *Shastra*= Science). Many of the drugs employed for *Rasayana Chikitsa* (rejuvenation therapy) contain mercury (Rasa) and are classified as *Rasaushadhies*.

However, demand of traditional or indigenous and complementary & alternative medicine (CAM) usage has grown spirally, signposting need of in depth research in its physical, biological and chemical nature of substances [2]. Now-a-days modern scientists are bothered about the presence of heavy metals in *Ayurvedic* preparations, and in tune with such calls there is a need of such studies. Indeed, to answer the number of research questions for contemporary results regarding efficacy concerns, help accommodate and encourage the successful translation of findings from lab to practice. Heavy metals are natural components have a density of 6.0 g/cm3 or more when compared with soil density 2.65 g/cm3 and occur naturally[3]. In Ayurveda, heavy metals like Copper, Zinc, and Lead, etc.used in divergent preparations, after transforming metal into non-metallic form [4].

The use of *Kajjali* in medicine started in 8th Century AD.3 during the period of *Nagarjuna* who was the pioneer of *Rasashastra*. However, the ingredients of *Kajjali* viz Parada and Gandhaka are therapeutically used since the period of *Charaka Samhita* and *Sushruta samhita* which are dated 7th -8th century B.C [5]. *Kajjali* is made by following the classical method of *Shodhanavidhi* and *Kajjalikarana*. Parada and Gandhak separately undergo *Shodhanavidhi* whereby they are expected to be purified, detoxified and potentized[6]. Mercury and sulphur thus processed are taken in equal weight and triturated till the shiny globules of Parada are no longer visible called as *Kajjali* [7]. *Kajjali* is a compound used in *Ayurvedic* therapeutics in a significant way. It is a prime ingredient of the majority of *Rasaushadhies* and there is a wide spread use of these *Rasaushadhies* for about 13 centuries. *Kajjali* cures many maladies by pacifying the tridoshas, and it is also used as *Vrushya* (an aphrodisiac), *Sahapana* (taking together with medicine), and *Anupana* (a vehicle taken after medicine)

The addition of sulphur in the drug is supposed to counteract the toxicity of mercury [8]. Thus to understand the safety and efficacy of this drug formulation it was felt necessary to characterize this *Ayurvedic* drug compound. Chemical constituents of *Swarna bhasma* (Gold ash) has been reported in a study[9]. Very few publication for complete characterization of *Samagandhak Kajjali* have been reported. So, in our study we aimed to evaluate the particle and chemical characterisation of *Kajjali* in conjunction with *In vitro* toxicity and osmotic fragility test.

2. Methodology

2.1. Raw Materials

Parada was procured from the Purple products private Ltd. Mumbai. Gandhak was purchased from Kahn Impex, Mumbai. Chemicals and reagents required for analytical work were procured from Sigma-Aldrich, Mumbai along with other local suppliers.

2.2.Experimental Animals

18 adult albino rats of wistar strain of both sex weighing between 150-200g were used in this study, procured from the Haffkine Biopharmaceutical Corporation Ltd, Mumbai. The animals were kept in standard conditions like 22 ºC ± 2 ºC and relative humidity 55 ± 15 %. Rats were freely accessible to food and water *ad libitium* with 12 hours light and 12 hours dark cycle. All animals were acclimatized for at least 5 days before start of the study. All the experimental protocols were approved by Institutional Animal Ethics Committe (IAEC) and performed according to the CPCSEA guidelines for the care and use of animals.

2.3. Preparation of Samagandhak Kajjali

Samagandhak Kajjali was prepared at manufacturing unit of Shree Dhootapapeshwar Ltd, Panvel from raw materials - Parada and Gandhak as per Ayurvedic text reference**:***Rasa-Tarangini* –6/107. Double distilled Parada obtained was purified by trituration with garlic Juice. For purification of the Gandhak, the traditional method using cow's milk was employed. In this method, Gandhak was heated up to its melting temperature and the resulting liquid was poured through a filter into a vessel containing boiled milk. Gandhak settled on the bottom and assiduosly milk decanted off. It was collected in another stainless steel container. Process was repeated thrice. Finally, washed with water and dried to get in purified form. Purified Parada and purified Gandhak added in equal quantity in the *Kajjali Patra* and triturated for 72 hours continuously at 30 rpm to get luster free *Kajjali.*

2.4. Methods

The composition and the structure of *Samagandhak Kajjali* using *X-ray diffraction* (XRD), *Scanning electron microscopy* (SEM), *X-ray photoelectron spectroscopy* (XPS), *Particle size analyzer*, *Thermo-gravimetry analysis* (TGA) and *X-ray fluorescence* (XRF) and *Atomic Absorption Spectroscopy* (AAS) have been performed.

2.5. Instruments and procedure involved

For powder X-ray diffraction (XRD), a Philips 1710 X-ray diffractometer with CuKα radiation (λ=1.5418 A^{Δ}) operating at 40 KV and 20 mA was used. Pattern was recorded for the angle (2θ) ranging from 5-70 degree at a scanning rate of 3 degree/second. X-ray photoelectron spectra (XPS) measurement was performed on KRATOS AXIS 165, using none monochromatized Al Kα X-ray as the excitation source. The sample was pressed in the form of pellet and placed in sample treatment chamber to get vacuum of the order of 2 x 10^{-6} Torr before placing the sample in to sample analyzing chamber to get vacuum of the order 10^{-8} to 10^{-9} Torr. The X-ray power supply was run at 15kV and 5mA and scanning performed.

Sample subjected to EDXRF (Energy dispersive X-ray Fluorescence) analysis to evaluate complete material balance. The samples in the form of powder were prepared as pellet in a boric acid matrix and subjected to XRF analysis. Elemental content present in the drug sample was reported. Quantitative determination of bulk elemental composition in the sample was carried out by EDAX, which was attached with Scanning Electron Microscope (SEM). For EDAX analysis, the sample was packed into a hole in an aluminum stub (9 mm diameter, 9 mm depth). The operating parameters were: 30 keV, count-rate 1,500 ± 500 counts/s, working distance 10 mm, chamber pressure set to <2.2 X 10^{-4} torr, tilt angle 0°, and accumulation time 50 s.

Elements in ppm level were estimated by Atomic Absorption Spectrophotometer (AAS) Perkin Elmer Analyst 400, USA. *Kajjali* samples were collected at different stages of trituration from *Kajjali Patra*. Particle size of these samples was checked by laser particle size analyzer. Scanning Electron Microscope (SEM) measurement was performed on JEOL ltd, Japan. The powder sample was placed in chamber with power supply at 10 kV scanning performed at different magnification ranging from 1000x to 3500x.

Thermogravimetry (TG) is based on continuous recording of mass changes of a sample of material, as a function of a combination of temperature with time. Thermograms were recorded in a Nitrogen atmosphere on a Mettler thermal analyzer TG-50. The sample was placed in an alumina crucible and the temperature was varied from 35-550 ºC with heating rate of 10 ºC/min.

2.6. *In vitro* lethality test

From past decades to now a days, toxicity of mercury and sulphur is a major concern due to it's organ toxicity, at a cellular level . Brine shrimp assay is considered as a impertinent tool for the estimation of preliminary detection of toxicity for several compounds. The procedure has been followed as discussed elsewhere [10]. However, calculated the percentage of mortality (% M) using the following formula:

% M = Percentage survival in the control – percentage survival in the treatment group.

2.7. Osmotic Fragility Test

Ashodhit and *Shodhit Kajjali* (4.5mg/200 gm) was administered orally to different groups of rats, daily for 21 days. Blood samples of all the animals were collected and in tubes containing anti-coagulant. It was centrifuged and equal quantity of 0.9% saline was added and again centrifuged. The supenatant so obtained was discarded and the pellet of RBCs thus obtained; was suspended in equal volume of saline solution. Certain volume of each of this was added to saline solutions of varying toxicity and observed for their haemolysis.

3. Results

3.1. X-ray powder diffraction (XRPD)

This technique was used for the characterization of compound through the crystalline phase identification. XRD pattern of *Kajjali* is shown in Fig. 1. Sample identification was done by matching d spacing with the standard JCPDS database (Table 1). XRD pattern shows HgS along with free sulphur are the crystalline phase present in *Samagandhak Kajjali*. HgS is present in cubic and hexagonal form. Free Sulphur present in ortho-rhombic form.

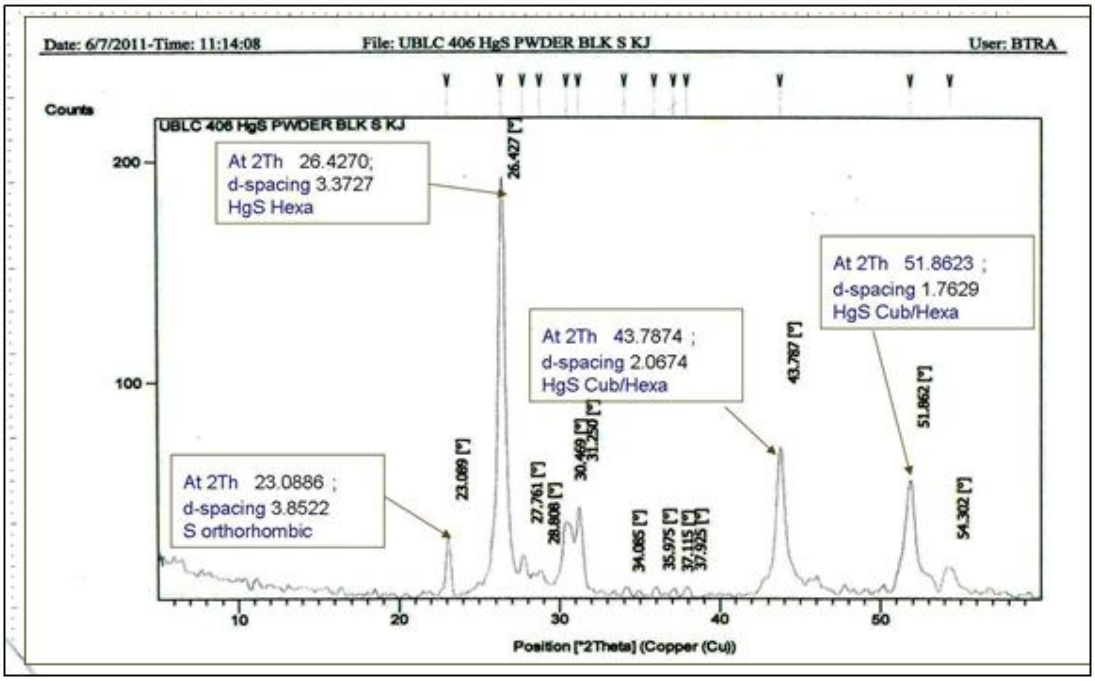

Fig 1: XRD – spectra of Samagandhak Kajjali

Table 1 d spacing of peaks of Samagandhak kajjali

Pos. [02Th.]	23.0886	26.4270	27.7607	28.8080	30.4688	31.2500	43.7874	51.8623	54.3020
dspacing [A0]	3.85227	3.37272	3.21364	3.09915	2.93390	2.86232	2.06749	1.76298	1.68940
Rel. Int. [%]	14.57	100.00	10.43	6.71	18.21	21.80	35.94	27.95	7.75
Crystal str	S-ortho rhom	HgS - Hexa	S-ortho rhom	HgS - Hexa	HgS - Cub	HgS - Hexa	HgS-Cub/Hexa	HgS Cub/Hexa	HgS – Hexa

3.2. X- ray Photoelectron Spectra (XPS)

This analysis provides valuable information for the surface state of the drug sample. A typical survey spectrum of the drug *Kajjali* confirming the presence of mercury and sulphur was observed (Fig. 2a). High resolution spectra at Hg core level showed the presence of the peaks at 100.52 eV and 104.62 eV corresponding to Hg (4f) Fig. 2b. S core level shows the presence of peaks at 161.74 eV and 162.88 eV corresponding to S (2p). Fig. 2c. In addition, it also shows the presence of C (1s) peak 284.62 eV as well as O (1s) peak at 532.54 eV (Fig. 2d and 2e). Presence of C and O, on the surface of the drug by XPS supports the idea of the coating of organic molecules on thesurface of the metallic compounds.

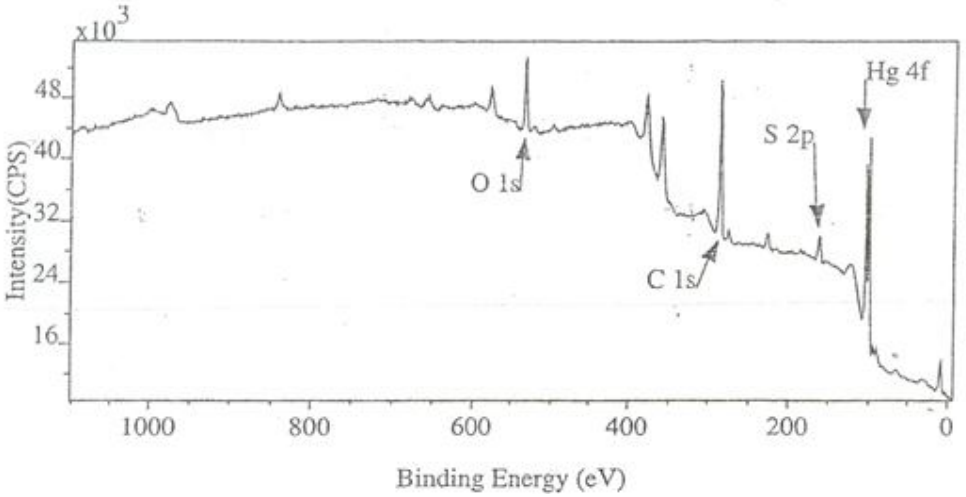

Fig 2a: XPS spectra of Samagandhak Kajjali

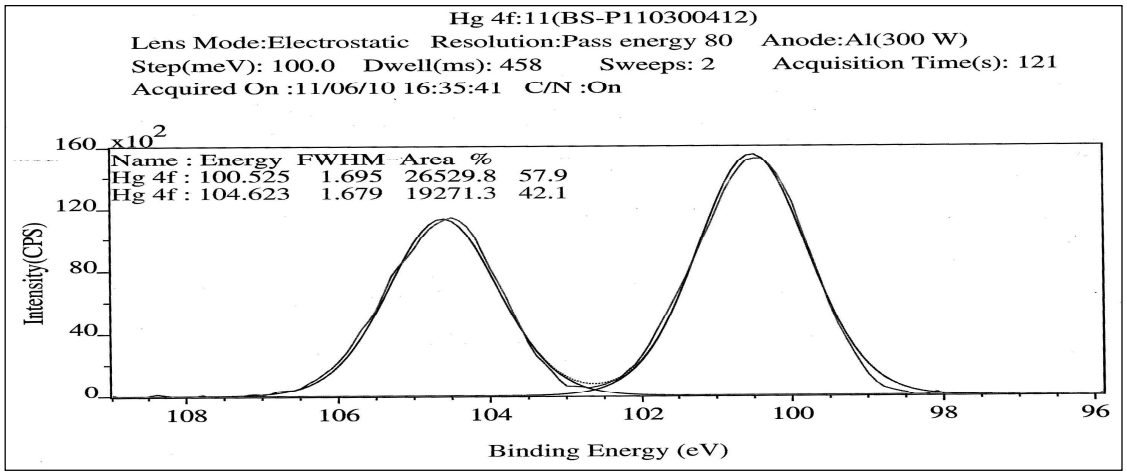

Fig 2b: Mercury (Hg) peak positions in XPS spectra of Samagandhak Kajjali, Hg4f peaks of Samagandhak Kajjali. High resolution spectra at Hg core level with peaks at 100.52 eV and 104.62 eV corresponding to Hg (4f).

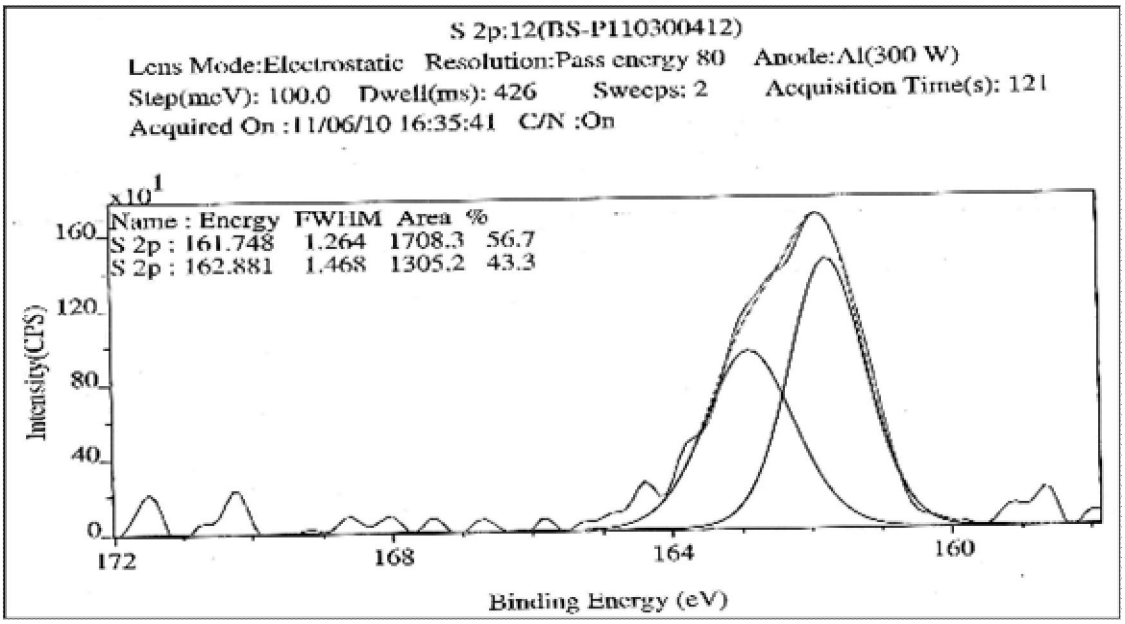

Fig 2c: Sulphur (S) peak positions in XPS spectra of Samagandhak Kajjali, S^2P peaks of Samagandhak Kajjali

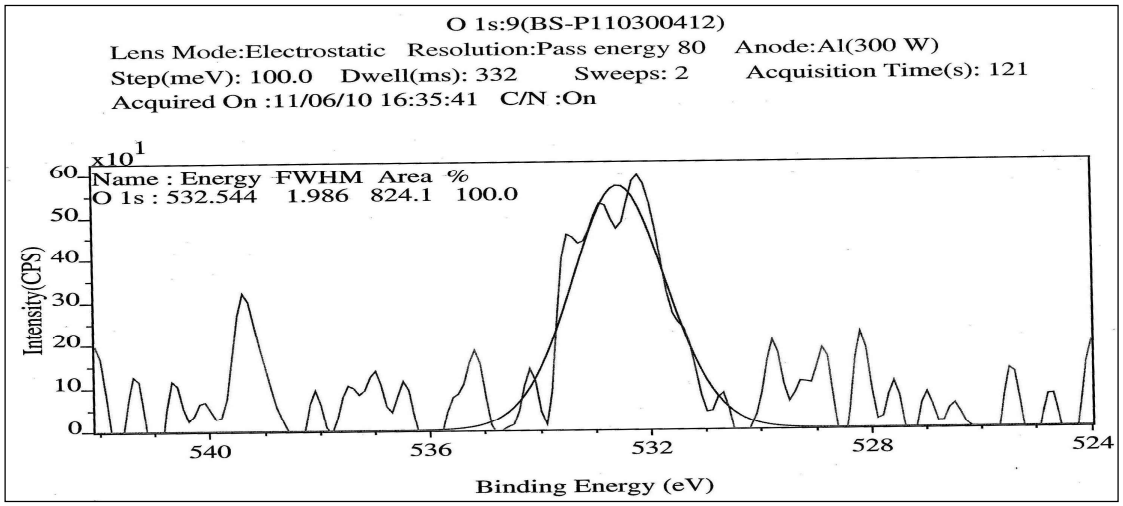

Fig 2d: Oxygen (O) peak positions in XPS spectra of Samagandhak Kajjali High resolution spectra of O (1s) core level with peak at 532.54 eV

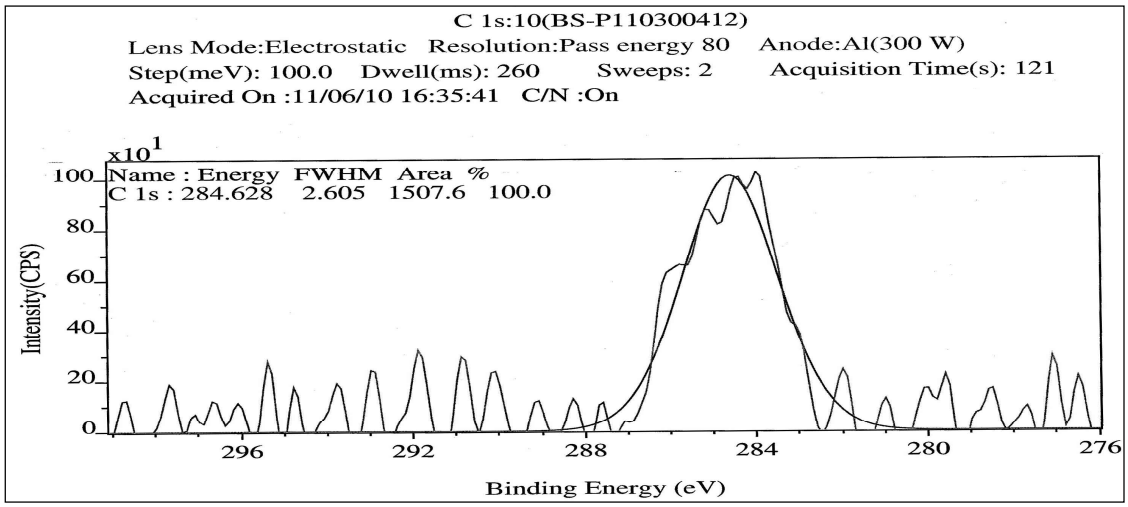

Fig 2e: Carbon (C) peak positions in XPS spectra of Samagandhak Kajjali High resolution spectra of C (1s) core level with peak at 284.62 eV

3.3. Particle size analysis

Particle size of these samples was checked by laser particle size analyzer. *Kajjali* samples were collected at different stages of trituration, i.e., in intermittent intervals from Kajjali Patra. Particle Size reduced significantly from 10 microns (After 12 hrs) to 1 microns (after 72 hrs) have shown in Table 2 & Fig. 3a-3f.

Table 2: Average particle size of Samagandhak Kajjali samples

S.No	Sample	Average Particle Size
1	Samagandhak Kajjali (After 12 hrs.)	9.66 (um) microns
2	Samagandhak Kajjali (After 24 hrs.)	2.59 (um) microns
3	Samagandhak Kajjali (After 36 hrs.)	1.3 (um) microns
4	Samagandhak Kajjali (After 48 hrs.)	1.15 (um) microns
5	Samagandhak Kajjali (After 60 hrs.)	1.26 (um) microns
6	Samagandhak Kajjali (After 72 hrs.)	1.06 (um) microns

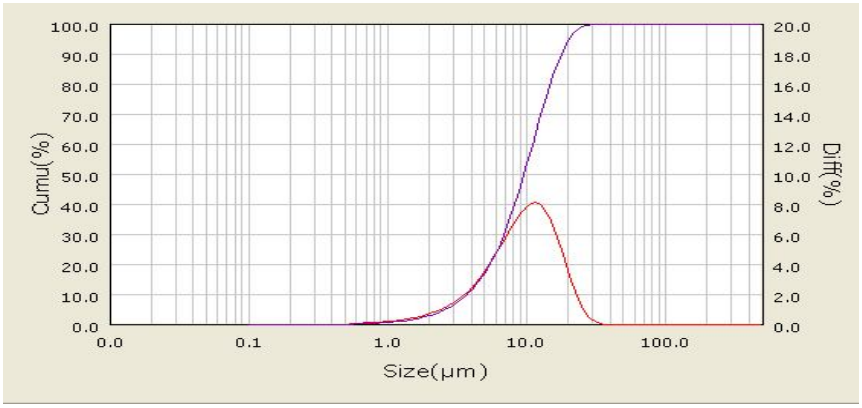

Fig 3a: Samagandhak Kajjali (After 12 hrs.)

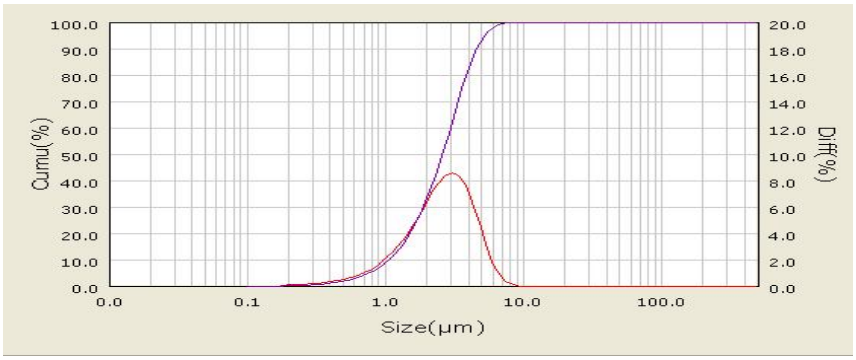

Fig 3b: Samagandhak Kajjali (After 24 hrs.)

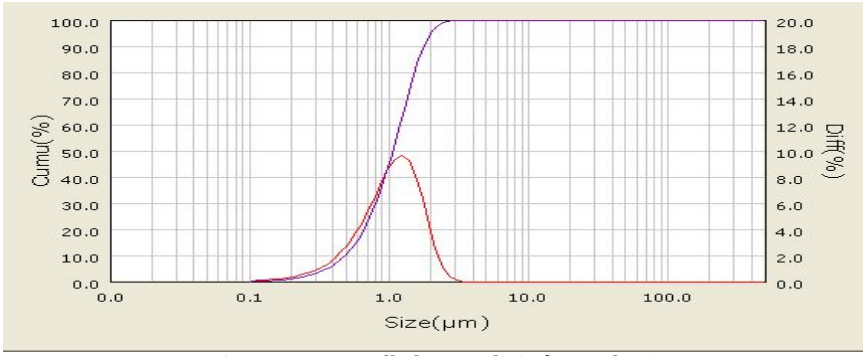

Fig 3c: Samagandhak Kajjali (After 36 hrs.)

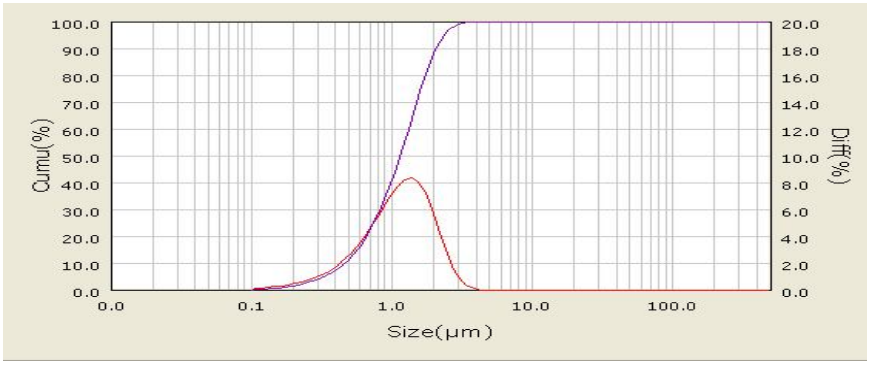

Fig 3d: Samagandhak Kajjali (After 48 hrs.)

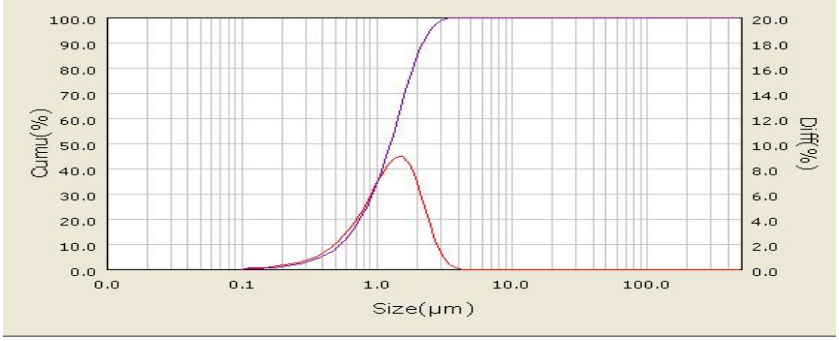

Fig 3e: Samagandhak Kajjali (After 60 hrs.)

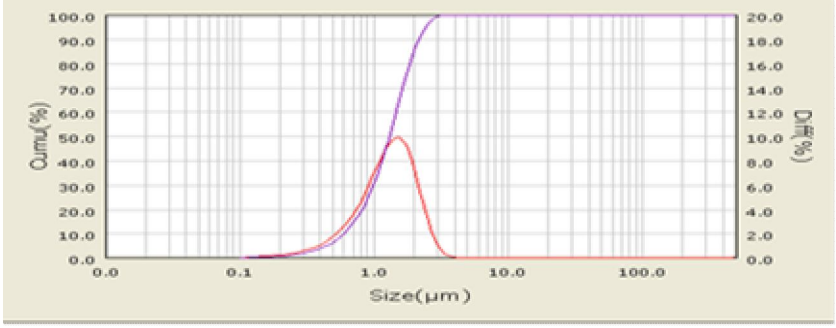

Fig 3f: Samagandhak Kajjali (After 72 hrs.)

3.4. Scanning Electron Microscopy (SEM)

SEM photomicrograph of *Samagandhak Kajjali* particles shows the appearance of particles of 10 um and less than 5 um size particles i.e. upto 0.23 um in size. SEM image of the drug sample shows cubic shape like structure with the particle size lying in the micro range. Particles with Rhombohedral features are also observed. From the image it is clear that several crystallites are agglomerated in a particle giving rise to microcrystalline structure as shown in Fig. 4a-4c.

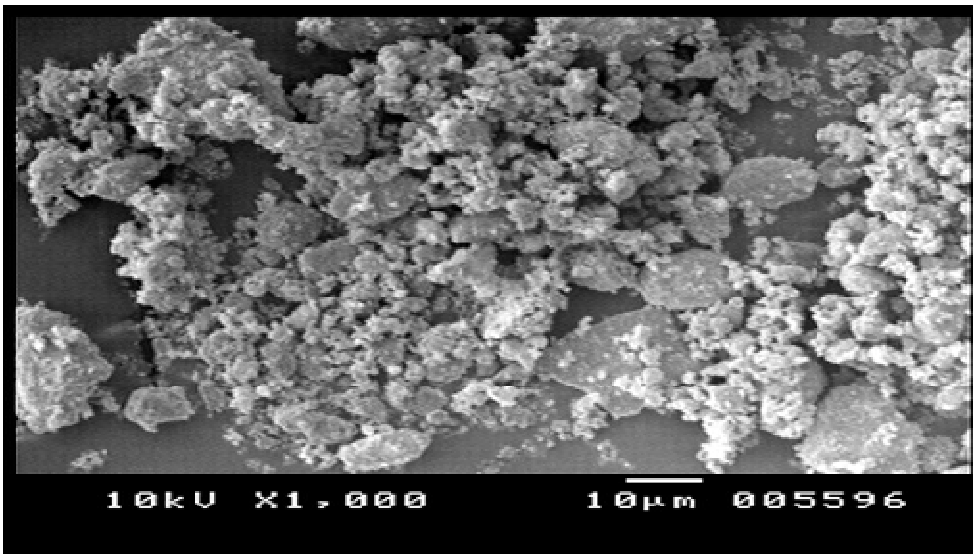

Fig 4a: SEM Photomicrograph of Samagandhak Kajjali at Magnification of 1000X (10kV)

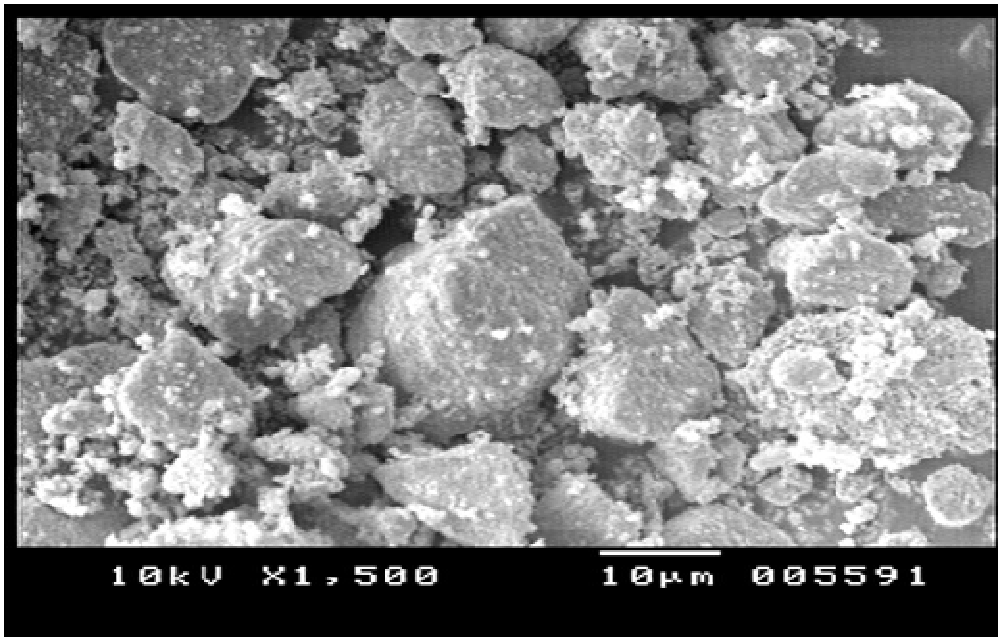

Fig 4b: SEM Photomicrograph of Samagandhak Kajjali at Magnification of 1500X (10kV)

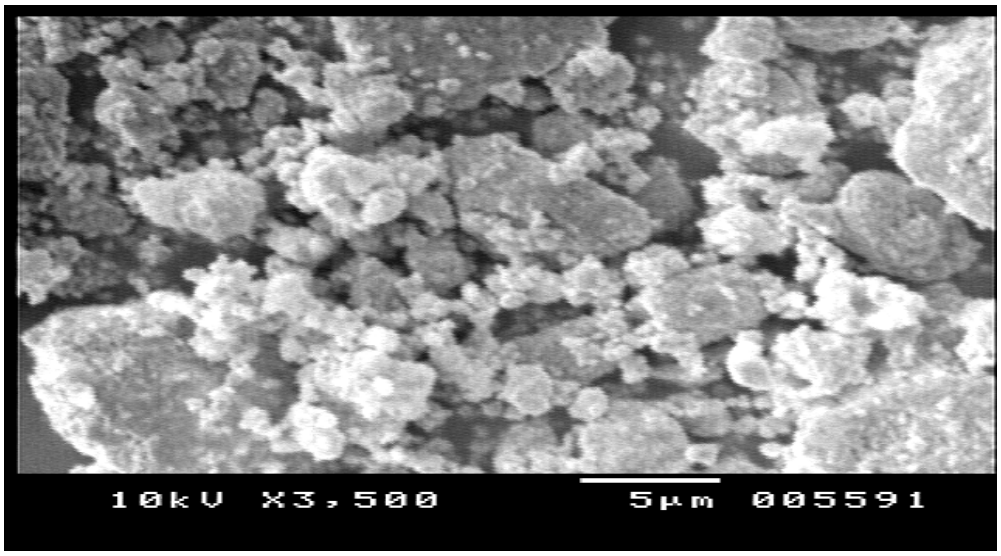

Fig 4c: SEM Photomicrograph of Samagandhak Kajjali at Magnification of 3500X (10kV)

3.5. Thermogravimetric Analysis (TGA)

In this method of analysis, different peaks of decomposition were observed, i.e., Step-1 at 35 to 170 ^{0}C with 0.5 -1.5 % of weight loss, step-2 at 170 to 320 ^{0}C with 40-45% of weight loss, and step-3 at 320 to 505 ^{0}C with 53-58% of weight loss, respectively (Fig.5).

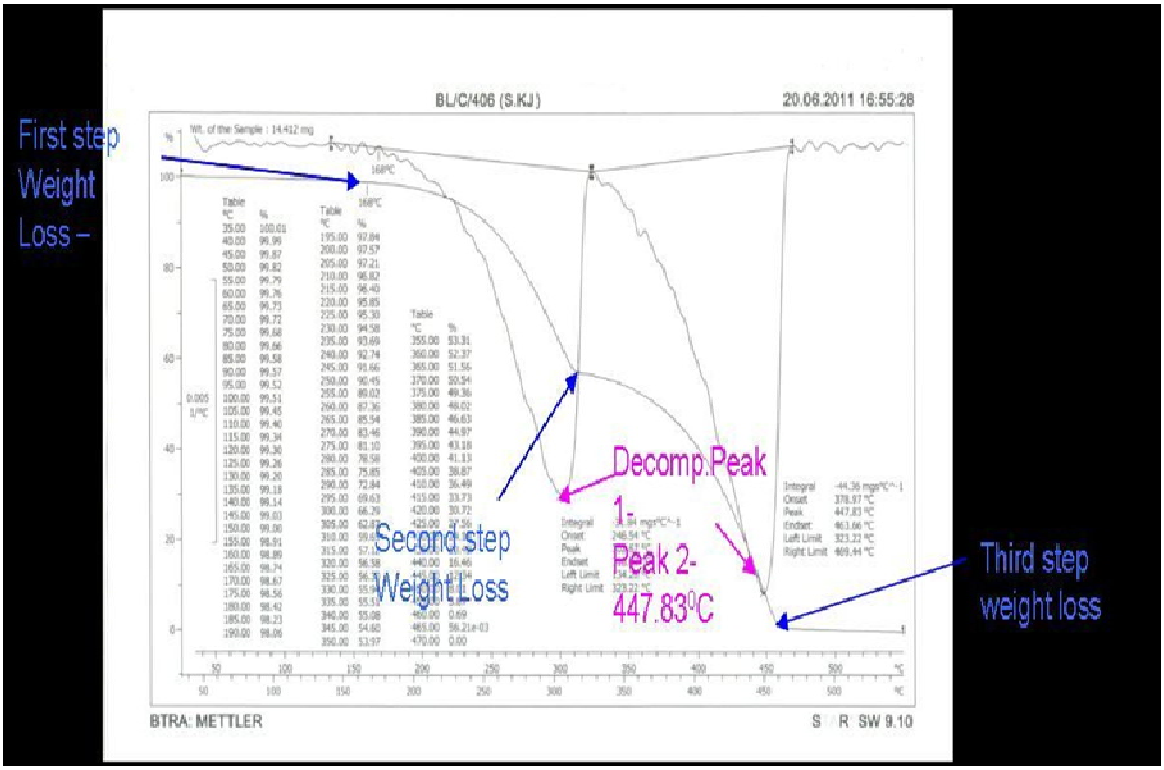

Fig 5: Thermagravimetric Analysis (TGA) of Samagandhak Kajjali

3.6. Energy dispersive X-ray Fluorescence (EDXRF)

EDXRF Analysis is to evaluate complete material balance. The samples in the form of powder were prepared as pellet in a boric acid matrix and subjected to XRF analysis. Each characteristic peak of the element compared with the standard energy levels and the elements were identified. Elemental content present in the drug sample was reported in Table 3. Mercury and Sulphur are major ingredients known to be present in the drug sample of *Samagandhak Kajjali*, this is followed by Phosphorus (P), Zinc (Zn), Selenium (Se), and Calcium (Ca) (Table 3).

Table 3: Material Balance of Samagandhak Kajjali by EDXRF & AAS

S. No.	Elements (in %)		Samagandhak Kajjali
			P110300412
1	Mercury	(Hg)	49.40
2	Selenium	(Se)	0.04 ppm
3	Calcium	(Ca)	0.02
6	Sulphur	(S)	49.90
7	Phosphorus	(P)	0.67
8	Zinc	(Zn)	12.39 ppm
Total composition (%)			**99.99**

3.7. *In vitro* Toxicity study

The percentage of larvae incase of *Shodhit Kajjali* was (92.30 %) and that incase of *Ashodhit Kajjali* (91.66 %) and control was (95 %). There is no significant ($p< 0.05$) difference observed in percent mortality (%) of Brine shrimp larvae in treatment group as well as control group.

3.8. Osmotic Fragility Test

The percentage of Sodium Chloride Solution (NaCl), which causes lyses of RBCs in the beginning was 0.50 % for control group of animals, 0.53% for *Shodhit kajjali* group of animals and 0.51% for *Ashodhit Kajjali* group of animals. The % concentration of NaCl solution, which causes complete haemolysis of RBCs was 0.35% for control, 0.26% for *Shodhit Kajjali*, and 0.30% for *Ashodhit Kajjali* group of animals. Lastly, osmotic fragility of RBCs was not affected significantly ($p< 0.05$) for the group of animals treated with *shodhit* and *Ashodhit Kajjali*.

4. Discussion

Traditional medicines are used in the treatment of various chronic disorders and for the improvement of well being an individual[11] . In *Ayurveda*, various compounds of Lead, Mercury, Arsenic etc. are used for various therapeutic process, after detoxification process by the various heat and cool cycles in oil, buttermilk, rice gruel, cow's urine, herbal decoctions, etc [4,12,13]. *Bhasmas* are unique preparations involving metallic/mineral preparartions

ignited for certain quantum of heat to transform to non-toxic organo-metallic form[14]. Though the metallic mercury is known to be toxic to the biological system, no compelling evidence has been put forth to suggest any toxic effects of this moeity of mercuric sulphide (*Kajjali).* This non-toxic effect may be attributed due to following factors-Preparation of *Samagandhak Kajjali* was carried out strictly as per Ayurvedic text reference. Shodhan of key ingredients viz. Mercury and Sulphur was performed and finally both the ingredients were mixed by trituration method to get the end product – *Samagandhak Kajjali.*The manufacturing process which invloves process reagents like garlic paste, milk, The trituration /grinding process for a long duration and heat invloved in preparation may be attributed to the change in the chemical nature of the raw material.

Garlic useful in purification of mercury as per ancient scripts. In general, organic components will burn out in the preparation of bhasmas (at the above 400 ^{o}C). XPS analysis shows the presence of Carbon and Oxygen in the sample and it explains the formation of organo-metallic complexes.

XRD pattern shows HgS along with free sulphur are the crystalline phases present in samagandhak kajjali. HgS is present in cubic and hexagonal form, 2θ position at 23.08, 26.42,27.76 28.80,30.46, 31.25,43.78, 51.86, and 54.30 with d-spacing of 3.83, 3.37, 3.21, 3.09, 2.93, 2.86, 2.06, 1.76 and 1.68 A^{o} respectively. Free sulphur present in ortho-rhombic form. Absence of free mercury is confirmed in XRD analysis. XPS spectra have shown the presence of Hg and S peaks confirming the presence of HgS compound on the surface along with free sulphur. Further, the presence of the C and O peaks on the surface of the drug proposes the concept that these organic matter acts as carrier and play role in drug delivery system.Thermogravimetric analysis findings highlights the decompositon a three step process which differentiates this compound from HgS.

Particle size analysis is a useful technique in a broad range of applications. Processing of purified sulphur and purified mercury in *Kajjali Patra* resulted in micronizing the material. This trituration process significantly reduced the particle size from 10 microns to less than 1 microns. Bioavailability of drug is understood to be dependent on its particle size. Rate of diffusion is proportional to the surface area and the particle size is inversely proportional to the rate of adsorption.

Moreover, particle size distribution is also known to be linked with physical and chemical properties of drug like stability, chemical reactivity and dissolution rates. Complete elemental assay of *Samagandhak Kajjali* performed by EDXRF, AAS showed presence of trace elements like Zn, P, and Se along with major elements- Hg and S. Zinc is useful for proper growth and immunity, selenium is useful as antioxidant. Several other elements were checked and found to be present in within WHO limits [15]. Trace elements detected in the product are due to the addition of herbal ingredients and can make the formulation bioavailable [9].

Garlic useful in various diseases like hyperlipidaemia, dyslipidaemia, in hypertension, fibrolytic activity and renoprotective [16]. Finally, it could be concluded that *Kajjali* present in mercuric sulfide (HgS) with additional sulphur with size of 1.06 (um) microns and associated with organic molecules like Carbon and Oxygen making it more biocompatible and non toxic at therapeutic level. Further scope of the study involves the instrumental analysis of Single Crystal XRD, Raman Spectra in addition with molecular modeling. EDXRF, AAS, XRD, XPS, Particle Size and TGA indicated that these methods can be used for rapid physiochemical fingerprints. XRD analysis of Samagandhak Kajjali confirm that it is mostly as mercuric sulphide with free Sulphur. Presence of Zn , P and Se in the formulation is conducive to healthy metabolism. Results reiterates that the Ayurvedic herbo-metallic compound is not similar in properties to chemical compound of synthetic origin because of the standard Ayuredic textual method with process reagents of herbal origin which contributes to its safety and therapeutic value.

5. Conclusion

By observing the results of structural and chemical characterisation of the study, clearly delineats in crystal view manner of its safety concern. But modern scientists claim that these preparations are toxic to health as they contain colossal amount of metal. Indeed, many factors like preparation based factors, chemical nature based factors, type of vehicle used, therapy associated factors, and pharmacological factors, etc, determine whether the traditional medicines are toxic or not. But, Ayurvedic preparations manufactured as per ancient scripts along with modern techniques are purely non-toxic. As there are no molecular targets available for treatment of Ayurvedic traditional preparations, and highly intensive research needed further to establish the appilcability in modern text by using physico-chemical, biological and in engineering point of view. The function of trace elements would help in understanding the pharmacological activity and its non-toxic effects. With complex nature of herbo-mettalic formualtions, such study can be used as a tool for quality control checks of *samagandhak kajjali.*

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5. References

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