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Pharmaceutico-analytical Study of Samaguna Kajjali with respect to different duration of trituration

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Abstract

Kajjali is a Sagandha, Niragni Parada yoga. The Bandha involved in this preparation is Kajjali Bandha, where purified Parada and Gandhaka are thoroughly ground in definite proportion, to get a fine black powder called Kajjali. Among Khalviya Rasayana, Kajjali is having prime importance as it forms base to many mercurial preparations. Though Ancient scholars mentioned some quality checks and end points in preparing Kajjali, it's not sufficient in the present scenario. It is found that Kajjali was prepared in different durations i.e. 76 hours, 130 hours, 255 hours and 280 hours for previous dissertation works carried out in the same institution. Apart from Kajjali siddhi lakshana, there is a need of more sophisticated testing methods for determining the physical and chemical changes of Kajjali at different duration of trituration.

Keywords: Parada; Gandhaka; Kajjali; Hingula

1 Introduction

Kajjali is a Sagandha, Niragni Parada yoga. The Bandha involved in this preparation is Kajjali Bandha[1], where purified Parada and Gandhaka are thoroughly ground in definite proportion, to get a fine black powder called Kajjali. Among Khalviya Rasayana, Kajjali is having prime importance as it forms base to many mercurial preparations. Though Ancient scholars mentioned some quality checks and end points in preparing Kajjali are not sufficient in the present scenario. On screening previous dissertation carried out in Taranath Govt Ayurvedic Medical College, Bellary-Karnataka, it is found that Kajjali was prepared in different duration i.e. 76 hours, 130 hours, 255 hours and 280 hours. Apart from Kajjali siddhi lakshana, there is a need of more sophisticated testing methods for determining the physical and chemical changes of Kajjali at different duration of trituration with uniform pressure.

Objectives

- Preparation of Samaguna Kajjali and their Pharmaceutico-Analytical study.
- To know the physical and chemical changes of Kajjali at different duration of mardana.
- To carry out Physico-Chemical analysis of Kajjali.

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2 Methodology

2.1 Materials

Genuine raw materials on the base of Grahya lakshana, were collected from authentic source. Necessary processing of raw material was done in Pharmacy section of P.G. Dept of Rasashastra, Taranath Government Ayurvedic Medical College, Bellary, Karnataka. Analytical studies / Instrumental analysis of Kajjali were carried out in established laboratories.

2.2 Pharmaceutical study

2.2.1 Preparation of Samaguna Kajjali

Table 1 Quantity of Parada and Gandhaka

Sl. No.	Ingredients	Chemical formula	Quantity
01	Shudha Parada	Mercury (HgS)	300gms
02	Shuddha Gandhaka	Sulphur (S)	300gms

2.2.2 Procedure

300gms of Hingulottha Parada was taken in Khalva and 300gms of finely powdered Shodhita Gandhaka was added and triturated. Trituration was done slowly with uniform speed till all the Kajjali Lakshanas[2] were observed i.e. the whole mixture converts into a fine, black, smooth, lusterless powder.

2.2.3 Precautions

- Dried and clean khalwa and peshani was taken.
- Gandhaka was finely powdered, before adding it to Parada.
- During trituration spilling of Kajjali from the Khalwa was avoided.

2.2.4 Observation

- All the Kajjali tests were performed and positive results were confirmed.
- Number of rotations per minute is 25-30.
- Final weight of the Kajjali after Kajjali Siddhi laxanas – 445gms.
- Loss of Kajjali – 35gms
- Total 120gms Kajjali was taken for samples.
- Total number of samples taken is 4.
- 30gms of Kajjali is taken for each sample.

3 Results

Table 2 Classical parameters for Analysis of SK at 76 hrs

Test	Observation
Varna	Black colour
Sparsha	Smooth and soft
Gandha	Slight Sulphur Smell.
Rekha Purnatva	Positive
Varitaratva	Positive
Nischandratva	Negative

Table 3 Classical parameters for Analysis of SK at 130 hrs

Test	Observation
Varna	Black colour
Sparsha	Smooth and soft
Gandha	Slight Sulphur Smell.
Rekha Purnatva	Positive
Varitaratva	Positive
Nischandratva	Negative

Table 4 Classical parameters for Analysis of SK at 255 hrs

Test	Observation
Varna	Black colour
Sparsha	Smooth and soft
Gandha	Slight Sulphur Smell.
Rekha Purnatva	Positive
Varitaratva	Positive
Nischandratva	Few shining particles are present

Table 5 Classical parameters for Analysis of SK at 280 hrs

Test	Observation
Varna	Black colour
Sparsha	Smooth and soft
Gandha	Slight Sulphur Smell.
Rekha Purnatva	Positive
Varitaratva	Positive
Nischandratva	Positive

Table 6 Organoleptic characteristics of SK

Parameters	SK 76 hrs	SK 130 hrs	SK 255 hrs	SK 280 hrs
Colour	Black	Black	Black	Black
Odour	Characteristic	Characteristic	Characteristic	Characteristic
Taste	Tasteless	Tasteless	Tasteless	Tasteless
Appearance	Fine powder	Fine powder	Fine powder	Fine powder

Table 7 Physico-Chemical standardization parameters of SK

Parameter	Results n=3 %w/w			
	SK at 76 hrs	SK at 130 hrs	SK at 255 hrs	SK at 280 hrs
Loss on drying	1.66	2.23	2.63	1.86
Total ash	99.80	100	100	100
Acid insoluble ash	NIL	NIL	NIL	NIL
Water soluble ash	99.80	100	100	100
pH	6.0	6.0	6.0	6.0

Table 8 Result of Chemical tests of SK- 4 samples

Contents	SK at 76 hrs (In %)	SK at 130 hrs (In %)	SK at 255 hrs (In %)	SK at 280 hrs (In %)
Total mercury	34.35	41.20	42.75	44.35
Mercurous mercury	13.25	11.45	9.22	8.44
Mercuric mercury	21.10	29.75	33.53	35.91
Free mercury	NIL	NIL	NIL	NIL
Total sulphur	42.70	34.45	32.50	30.90
Free sulphur	1.25	1.15	0.40	0.20
Sulphite	31.20	26.55	24.40	26.11
Sulphate	10.25	6.75	6.70	4.59

3.1 X-Ray Diffraction Results

Table 9 XRD of SK at 76 hrs

Sample	Compound name	Chemical formula	Crystal structure
SK at 76 hrs	Metacinnabar	Hg S	Cubic

Table 10 XRD of SK at 130 hrs

Sample	Compound name	Chemical formula	Crystal structure
SK at 76 hrs	Metacinnabar	Hg S	Hexagonal

Table 11 XRD of SK at 255 hrs

Sample	Compound name	Chemical formula	Crystal structure
SK at 76 hrs	Metacinnabar	Hg S	Hexagonal

Table 12 XRD of SK at 280 hrs

Sample	Compound name	Chemical formula	Crystal structure
SK at 76 hrs	Metacinnabar	Hg S	Hexagonal

Table 13 SEM-EDX of raw Hingula

Element	Weight %	Atomic %
S	15.47	53.38
Cu	0.00	0.00
Ag	0.00	0.00
Au	0.00	0.00
Hg	84.53	46.62

Table 14 SEM-EDX of SK

Elements found	Concentration in percentage (%)			
	SK at 76 hrs	SK at 130 hrs	SK at 255 hrs	SK at 280 hrs
O	2.92	0	0	0
S	19.88	17.43	22.79	29.43
Hg	77.20	82.57	77.21	70.57

Table 15 Particle Size of SK- 76hr, 130hr, 255hr, 280hr

Effective diameter			
Sample	Mean diameter(nm)	Standard error	Effective diameter(nm)
76 hrs	669.5	75.2	630.5
130 hrs	450.1	80.3	424.5
255 hrs	412.6	63.9	424.8
280 hrs	371.4	61.0	319.4

Table 16 Observations of NPST of SK at 76 hrs

Sample Name	Phase I	Phase II	Phase III
SK at 76 hrs	Immediate drop was of orange spot with white margin fallowed by brown periphery ring.	Central orange spot faded (Slight whitish) and Outer brown periphery also faded.	Central white spot remained as it is. Orange spot became very dark and prominent forming the outer margin of the spot. Brown periphery faded gradually after 20 min. After 24 hour Brown periphery completely disappeared leaving white colour in its place.

Table 17 Observations of NPST of SK at 130 hrs

Sample Name	Phase I	Phase II	Phase III
S.K at 130hr	Immediate drop was of orange spot with white margin fallowed by brown periphery ring	Central orange spot faded (Slight whitish) and Outer brown periphery also faded.	Central white spot remained as it is. Orange spot became very dark and prominent forming the outer margin of the spot. Brown periphery faded gradually after 20 min. After 24hour Brown periphery completely disappeared leaving white colour in its place.

Table 18 Observations of NPST of SK at 255 hrs

Sample Name	Phase I	Phase II	Phase III
S.K at 255hr	Immediate drop was of orange spot with white margin fallowed by brown periphery ring	Central orange spot faded (Slight whitish) and Outer brown periphery also faded.	Central white spot remained as it is. Orange spot became very dark and prominent forming the outer margin of the spot. Brown periphery faded gradually after 20 min. After 24 hour Brown periphery completely disappeared leaving white colour in its place.

Table 19 Observations of NPST of SK at 280 hrs

Sample Name	Phase I	Phase II	Phase III
S.K at 280hr	Immediate drop was of orange spot with white margin fallowed by brown periphery ring	Central orange spot faded (Slight whitish) and Outer brown periphery also faded.	Central white spot remained as it is. Orange spot became very dark and prominent forming the outer margin of the spot. Brown periphery faded gradually after 20 min. After 24 hour Brown periphery completely disappeared leaving white colour in its place.

Table 20 Calculation of the Pressure

	Weight of pestle *	Normal load**	Maximum load***
Weight of pestle in(kg)	4.6	6.1	8.6
Weight in grams	4600	6100	8600
Area $=\frac{\pi}{4}D^2$			
π	3.142	3.142	3.142
Diameter in cm	33	33	33
Square diameter (D^2) in cm^2	1089	1089	1089
π/4	0.7855	0.7855	0.7855
Area in cm^2	855.4095	855.4095	855.4095
1 *Newton* is equal to 9.81kg *1* N/cm^2 is equal to 9.81kg/cm^2			
Pressure=$\frac{force}{Area}$	0.005377541 kg/cm^2	0.007131088 kg/cm^2	0.01005366 kg/cm^2
	5.3775414 g/cm^2	7.131087508 g/cm^2	10.05366436 g/cm^2

4 Discussion

According to modern chemistry, intimate contact of Mercury and sulphur causes the chemical reaction and a compound Mercuric sulphide (HgS) is formed. According to the law of definite proportion, the compound HgS is formed by combination of Mercury and Sulphur in the proportion 6: 1. As compared to this modern concept, the proportion of Gandhaka is much higher in the Ayurvedic formulations of Parada and Gandhaka. In many preparations, special Kajjali is prepared by using Gandhaka in 1:1, 1:2, 1:3 or even 1:6 proportions. Even though the formulae of such compounds are HgS, this excess of Sulphur changes their properties.

Classically prepared Kajjali eventually acts as GI stimulant[3], locally also acts as neuro chemical irritant for intestinal mucosa. Acts as a catalyst and hence through its catalystic activity, better absorption of remaining herbal pharmacological molecules is also augmented. The net resultant activity of Kajjali in any khalvi rasayana increases the bioavailability of ingested drugs. The invariable addition of Kajjali in various herbal powders along with trituration under pressure will also make enhanced storage, viability, half life of the respective herbs.

In various prospective clinical observations, it is noted that the drug effect is more after the addition of micro quantum of well-prepared Kajjali. It is also helpful in reducing the dosage of herbal compounds. Kajjali compounds in GI tract undergo the process of adsorption and hence it also acts as GI stimulant. Pharmacological activity of such a compound can be still better observed. Effective dose of active drug when consumed will be gradually released, adsorbed and later on acts as per the requirement at target cell.

When the plasma concentration of effective drug declines again acceleration of release of active drug takes place through biofeed mechanism. In between these two phases the inert drug – Kajjali will be slowly stimulating the local membrano – enzymatic axis, means, effective drug dose decline is followed by inert drug release which when declines, again active drug is bio chemically signaled for release. Another pharmaco therapeutic advantage of Kajjali kalpa as per reverse pharmacological observation is, when compared to other pharmacological forms, the herbo mineral compound classically prepared by using Kajjali possesses large stay in intestinal mucosa[4].

Rasa shastra doctrines say that once the Kajjali kalpa enters the GI tract, it makes a separate coat and binds to the mucosal wall and stays for long time and produces pharmacological sustained rejuvinative effects5. Large stay of compactly layered Kajjali compounds possesses more enzymatic activity. The more the drug stays and more enzymatic activity is taking place then it is also realized that the drug takes more pushed entry through GI tract barriers. The long alarming of the intestinal Kajjali compound better crosses BIB (Blood intestinal barriers) and hence more pharmacological activity is possible by using Kajjali rasayanas[5].

Whereas addition of Kajjali in a herbal product, due to long stay – more enzymatic activity – more utilization of the active drug results in least or no drug loss. Kajjali kalpa has unique character of incremental dosing of micro molecules and hence a chance of drug reactions is reduced. This phenomenon is an advantage ahead with Kajjali kalpas.

It is to be noted that, abnormal hepatic metabolism may result in the persistence of any drug metabolites that act as antigens or have direct cellular toxicity.[5] The classically prepared Kajjali kalpa has this safe guard mechanism. The Kajjali / khalvi Rasayana doesn't allow abnormality to develop in hepatic metabolism as they are dhatvagni modulators. The theories of khalvi rasayana take care of drug metabolites in such a way that for the body they will not act as antigens. Hence Kajjali yukta herbo mineral products are devoid of drug reactions which are another contribution of clinical and reverse pharmacological observation. Of course Kajjali kalpas are clinically safely tried in the management of drug reaction itself.

Secondary to various infections and host cell immune mechanisms, polyclonal increase of immunoglobulin including IgE and increase in circulating immune complexes takes place[5], in various immunological problems, the administration of Kajjali kalpas is beneficial as it has anti IgE mediated reaction activity. It also scavenges circulating immune complexes due to long stay, sustained release and incremental dosing properties. Drug reactions can occur to the administered drugs. Hepatic and hematologic abnormalities can stem from immune mediated injury and cell toxicity.

The rasayana effect of Kajjali kalpas have proven immune enhancing effect, cellular rejuvinative effect systemic detoxification and antioxidant effects. Therefore by immuno enhancing activity and target cell protection activity the Kajjali kalpas minimize hepatic and haematologic abnormalities.

Any herbal powder when stratified with khalvi rasayana method will become a new complex chemically. The shelf life of the herbal active chemical molecules will be maintained for longer period due to the inert Kajjali effect. Enhancement of shelf life of herbs in Kajjali kalpas may be due to the compact stratification of black sulphide of mercury, opening multiple bonding capacity of processed mercury. Shelf life maintenance, enhancement and modulation are definitely to be magic bondage of mercury.

It is also worth to note that, when compared to the herbal powders alone, the half-life of Kajjali kalpa is more. Kajjali stratified compact herbal molecules will be benefited with prolonged half-life, hence the gradual and sustained drug plasma availability. To be still specific, Kajjali also enhances the half-life of herbal powders; Hence a technological natural advantage.

Sulphides of mercury are bactericidal and bacteriostatic. When Kajjali kalpas are administered, probably GI antimicrobial and bactericidal effects take place. When topically used the same will be dermatoprotective, antifungal antimycotic and antiseptic.

4.1 Discussion on XRD

The X-ray Diffraction studies were done at MIT, Manipal. Phase analysis of SK at 76 hrs, 130 hrs, 255 hrs and 280 hrs were done to clear out the structure and chemical composition of the samples.

4.2 SK at 76 hrs

The XRD Peaks of SK at 76 hrs showed totally 13 peaks at different angels (2θ) from 22.86 to 72.07. Out of 13 peaks 5 strong peaks were matching with standard peak values of HgS. These peaks of SK at 76 hrs sample which were compared with standard D-space JCPDS values confirmed that the presence of Metacinnabar (HgS) in Cubic crystal system.

4.3 SK at 130 hrs

The XRD Peaks of SK at 130 hrs showed totally 13 peaks at different angels (2θ) from 22.78 to 72.01. Out of 13 peaks 5 strong peaks were matching with standard peak values of HgS. These peaks of SK at 130 hrs sample which were compared with standard D-space JCPDS values confirmed that the presence of Meta cinnabar (HgS) in Hexagonal crystal system.

4.4 SK at 255 hrs

The XRD Peaks of SK at 255 hrs showed totally 13 peaks at different angels (2θ) from 22.83 to 69.84. Out of 13 peaks 5 strong peaks were matching with standard peak values of HgS, These peaks of SK at 255 hrs sample which were compared with standard D-space JCPDS values confirmed that the presence of Metacinnabar (HgS) in Hexagonal crystal system

4.5 SK at 280 hrs

The XRD Peaks of SK at 280 hrs showed totally 14 peaks at different angels (2θ) from 22.76 to 69.75. Out of 14 peaks 5 strong peaks were matching with standard peak values of HgS, These peaks of SK at 280 hrs sample which were compared with standard D-space JCPDS values confirmed that the presence of Metacinnabar (HgS) in Hexagonal crystal system. XRD of all the four samples were analyzed among them 76^{th} hr sample showed metacinnabar with cubic crystal structure but rest of three samples showed metacinnabar with hexagonal structure.

4.6 Discussion on EDX

SEM EDX reveals the accurate elemental identification and quantitative compositional information.

- The major elements present in S.K in 76 hours are O,S and Hg
- The major elements present in S.K in 130 hours are S and Hg
- The major elements present in S.K in 255 hours are S and Hg
- The major elements present in S.K in 280 hours are S and Hg

The percentage of mercury in SK at 76 hours, 130 hours, 255 hours and 280 hours are 77.20%, 82.57%, 77.21%, 70.57% respectively.

The percentage of Sulphur in SK at 76 hours, 130 hours, 255 hours and 280 hours are 19.88%, 17.43%, 22.79%, 29.43% respectively.

The percentage of Oxygen in SK at 76 hours, 130 hours, 255 hours and 280 hours are 2.92%, 0%, 0%, 0% respectively.

There may be chance of oxygen combined with mercury and forms the oxides of mercury. But in present study only in one sample i.e. 76th hrs presence of oxygen was found. Oxygen is absent in rest of three samples.

As the Trituration time increase the particles lose the spherical morphology and a

Transformation to irregular, prismatic, smaller particles can be observed. In samples obtained at Trituration time for 76 hrs, several particles containing Hg, sulphur and oxygen were observed.

These particles contain 77.20% of Hg, 19.88% of sulphur and 2.92% of oxygen, thus Indicating the partial oxidation of mercury. Gibbs energy for the formation of HgO is 13.94 kcal/mol at 298 K, which is lower than that corresponding to formation of HgS. Several authors describe the use of inert atmosphere, (Riviere-Huc et al. 2006 and Oji 1998), the addition of sodium sulphides and other compounds (Fuhrmann et al. 2002) with the aim of avoiding the oxidation of mercury.

Through EDX study of major elements present in the Kajjali is Hg and S.

4.7 Discussion on Particles Size by Zeta potential Analyzer:

- Mean Particle size of S.K at 76hr is – 669.5nm
- Mean Particle size of S.K at 130hr-450.1nm
- Mean Particle size of S.K at 255hr-412.6nm
- Mean Particle size of S.K at 280 hrs-371.4nm

The gradual reduction in the particle size of the SK at 76,130,255 and 280 hrs are due to mardana. The need for particle size control in the manufacture of pharmaceuticals is becoming increasingly apparent for the following reasons:

Particle size of a drug is of great importance in the transport from GI tract to the site of action by the way of blood and lymph. Because of much greater absorptive area available to molecules, the opportunity for molecules to penetrate the cell membranes is obviously higher. Particle size reduction will result in precise drug delivery and thereby increasing the bio availability of the drug. The particle size has an important influence on dissolution rate. Smaller the drug particle size larger the surface area, leads to faster dissolution.

4.8 Concept of Micromeritics

Micromeritics is the science and technology of small particles. The size and the surface area of particle can be related to the physical, chemical and pharmacologic properties of drugs. Clinically, the particle size of a drug can affect its release from dosage forms. Micromeritic property of a particle influences the physical stability. Smaller the particle size, better the physical stability of the dosage forms.

4.9 Discussion on pressure calculation

- Pressure is inversely proportional to area and directly proportional to force.
- This indicates that pressure applied through pestle is related to the surface area of the mortar.
- In this study an attempt has been made to find out the amount of pressure applied during trituration.
- For calculation of the pressure applied diameter and weight of the peshani and length and breadth of khalva and its surface area are considered and pressure is calculated through ANSYS Software.
- Pressure = $\frac{force}{area}$
- The weight of the pestle, normal load and its maximum load used for the preparation of Kajjali are 4.6kg, 6.1kg and 8.6kg respectively.
- Pressure applied at normal load and maximum load were 5.37g/cm2, 7.13g/cm2 and 10.05 g/cm2 respectively.
- Pressure distribution inside the mortar is also calculated.
- Throughout the Kajjali preparation uniformity of pressure and rotations was maintained.
- Pressure applied per cm2 area of the khalwa during single rotation in a normal load is 7.13g/cm2.
- So analysis of the pressure exerted helps for duration of the trituration. Because pressure differs from person to person.
- At least some attempt is made for the determination of pressure with the help of obtained data.

5 Conclusion

- Total four samples were taken for analysis during the trituration at 76th hr, 130th hr, 255th hr and 280th hr based on previous dissertation works of the same P.G department to know the physical and chemical changes of Kajjali apart from siddhi lakshanas.
- Rekhapurnatwa and Varitara were positive in SK at 76hr, 130hr, 255hr and 280hr samples.
- Nischandratwa is positive only in fourth sample i.e.at 280th hours.
- Total mercury values of SK at 76 hours, 130 hours, 255 hours and 280 hours are 34.35%, 41.20%, 42.75% and 44.35% respectively.
- The values of mercuric mercury at 76 hours, 130 hours, 255 hours and 280 hours are 21.10%, 29.75%, 33.53% and 35.91% respectively.
- The value of Mercurous mercury in SK at 76 hours, 130 hours, 255 hours and 280 hours are 13.25%, 11.45%, 9.22% and 8.44% respectively.
- The values of total sulphur in SK at 76 hours, 130 hours, 255 hours and 280 hours are 42.70%, 34.45%, 32.50% and 30.90% respectively.
- Free sulphur values in SK at 76 hours, 130 hours, 255 hours and 280 hours are 1.25%, 1.15%, 0.40% and 0.20% respectively.
- X-ray diffraction reveals that SK at 76 hrs is having Metacinnabar in cubic crystallinity form. But rest of the three samples is having Metacinnabar in Hexagonal form.
- EDX report shows that only SK at 76hr is having the major elements like O, S and Hg. But rest of the three samples shows only S and Hg elements.
- Particle size chronologically decreased in SK at 76 hours, 130 hours, 255 hours and 280 hours & they are 669.5nm, 450.1nm, 412.6nm and 371.4nm respectively.
- NPST result shows that there is no much change in the four samples.

ANSYS Software revealed that on and average pressure exerted during mardana was 7.13g/cm^2.

Compliance with ethical standards

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Disclosure of conflict of interest

No Conflict of interest

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