1. INTRODUCTION

1.1 Background

Bhasma are the medicines made from metal, mineral or marine products. They have a particle size of submicron to nano units for the purpose of proper absorption by the body. Raw metal/mineral/marine substances are converted into therapeutic form through processes that involve repeated incineration and grinding with certain herbs and other specified matters.\textsuperscript{1, 2}

In Ayurveda a single drug can be used for many conditions. In different conditions same drug acts differently. Many popular Ayurvedic drugs such as Swarna Bhasma, Kaseesa Bhasma etc. have many indications. Shodhita Swarna, Swarna bhasma, Shodhita Kaseesa and Kaseesa Bhasma are indicated in female reproductive system.

To quote examples

**Shuddha Swarna guna**

- Vishuddha kanakam ghrustham Vishabadha vinashanam|

  Madhuram Sheetalam Netryam **Garb hastapanam** uttamam  (Ref: RT 15/28)

Shuddha swarna paste prepared by rubbing with water or any prescribed dravadravya is having the properties like Madhura, sheetala, Netrya and **Uttama Aushadha for garb hastapanana.**

**Swarna Bhasma Guna**

- Mruta Swarnam Madhuramcha Vrushyam Hridhyamcha Netryam paramamcha medhyam | Rasayanam **Pumsavanopayogi** Vishapaham kantikaramcha shastam||

  (Ref: RT 15/69)
Swarna bhasma is having the properties like Madhura, Vrushya, Hridhya, Netrya, Medhya, Rasayana, **Pumsavanopayogi**, Vishanashaka and Kantikaraka.

- **Nagakeshara churnena suvarnam parishilitam|**
  
  Rutaou simantininantu **garbhadharanam uttamam|** (Ref RT15/105)

Administration of Swarna Bhasma along with Nagakeshara during rutukala helps for garbhashapana.

In the Indian system of medicine, Swarna bhasma preparations are highly valued and extensively used for the purpose of rejuvenation and revitalisation. Gold preparations have exhibited analgesic effects in rats and mice. Kamboj V P et al detected antitesticular effects of some metallic earth salts including gold chloride in rats and mice.

A clinical study by Dr B. Shreenivas Prasad on Swarna Bhasma has shown increased sperm motility from 25% to 54.5% and brought about a marked reduction in oligospermia, when given in the dose of 8mg per day for a month.

Study done by Sharma D. C et.al reported that Swarna containing Ayurvedic drug showed stimulating effects in impotent subjects. Other studies done by Chattopadyay et al on the effect of copper chloride on immature male rats indicated some stimulatory effects on gonad and observation on the gold chloride on immature male rats have shown significant stimulatory effects on testicular activities. AlokChattopadyay et al have conducted a study on gold chloride on stimulation of reproductive function in female albino rats showed stimulatory effect in epithelial cells and ovarian stroma with Graafian...
follicle and ovum in animals. There are few classical as well as patent compound formulations containing Swarna Bhasma are available in the market being used for the treatment of gynecological disorders.

Kaseesa Bhasma is another preparation which is widely used in many Ayurvedic preparations. Chemically, it is known as Ferrous Sulphate (FeSO₄·7H₂O). We come across the use of Kaseesa in brihatrayees and Rasa Shastra Texts for the female reproductive system.

To quote example

Shuddha Kaseesa Guna

- Kaseesam tuvaram Grahi vipako ushnatu sheetalam|
  Shwitragnam netram tualm vishagnam kacharanjanam||
  Vatashleshma amayaharam mutrakruchra prashanam|
  Kandu Pandu Krimignamcha Raktaranjanam Param||

Rajaha Pravartakam Balyam Jwaragnam Pleeha Nashanam|
Bahya prayoge vidneyam sankochakaram param||

(Ref: R T 21/232, 33)

Shuddha Kaseesa is Grahi, Ushna in vipaka, Sheeta in guna, Shwitra nashaka, Netrya, Vishanashaka, Ranjaka, Vata, Kapha roga, Mutra Krrichra, Kandu, Pandu, Krimiroga nashaka. Raktaranjaka, Rajaha Pravartaka, Balya, Jwarahara, Pleeha nashaka. Its external application works as Sankochaka.

Kaseesa Bhasma Guna is similar to Loha Bhasma. (Ref: RT 21/260)

Sharreerika rujam cha ati mansam va shannaataam|

Rajaha Kshaya samudbhutam Vardhakyaam Nashayed dhruvam||
Introduction

Kaseesa bhasma guna are similar to louha bhasma, both bhasma does shareerika ruja, manasika vikara, **Rajaha kshaya** and jara vyadhi nashana.

Shuddha Kaseesa and Kaseesa bhasma are used as Rajapravartaka\(^{11}\) (**Emmenagogue** - a drug or agent inducing or increasing menstrual flow) and one of its most popular preparations is Rajapravartini-Vati which is recommended in amenorrhea. Considering its **Emmenagogue** property an effort in this study has been made to assess the effect of Kaseesa Bhasma on reproductive function in female rats.

Though single bhasma are indicated in the treatment of many conditions but always in clinical practice we prefer to use compound formulations. So, in the present study along with Swarna Bhasma & Kaseesa Bhasma, additionally one of compound formulation of each bhasma available in the market were also studied. The compound formulation of Kaseesa Bhasma selected was Rajapravarthini Vati and compound formulation of Swarna Bhasma selected was Falaa Gold.

Based on the above classical as well as research updates present study was planned to assess the effect of Swarna Bhasma, Kaseesa Bhasma and their compound formulations on reproductive system of female albino rats.
1.1.1 OBJECTIVE

Main Objective
To study the effect of Swarna Bhasma and Kaseesa Bhasma on reproductive function in female rats.

Additional Objective
To study the effect of Swarna containing compound formulation and Kaseesa containing compound formulation on reproductive function in female rats.
1.2 REVIEW OF LITERATURE

1.2.1 Swarna

Name in different languages
Sanskrit – Suvarnam
Hindi - Sona
English - Gold
Latin - Aurum
Symbol - Au

History of Swarna:
Vedic Period: For centuriesGold has attracted the attention of the world for its multifarious uses. Rigveda, the oldest scripture of the world has references to Swarna. Whereas Rigveda mentions just the name ‘Swarna’ the other three Vedas elaborate its uses.

Upanishad: The Upanishad’s have cited several references to Swarna. The Ishavasyopanishad states "Hiranyamayenapatrenasatyasyapihitammukham|" (Ishavasyopanishad)

This means Swarna shows presence of satyamukha in hiranyamayapatra. It means in hiranyamayapatrasatyamukha is present. The rising sun resembles Swarnapatra. Sunrise destroys asatya. Many such references available in the Upanishad assert the importance of Swarna.

ShatapataBhrahmana: The Shatapata Bhrahmana mentions. Swarna as Agnivirya. The book gives a vivid account of utpatti of Swarna. According to which Jalamaithuna and Agni lead to agniviryaksharana which inturn lead to the utpatti of Swarna.
**Samhita Period:** During Samhitakaala Swarna was used to prepare Patra, JivhaLekhana, DhatuPatra, BastiNetra etc. In Charaka Samhita the properties of gold and its compound formulations such as LauhaRasayana are mentioned. Sushruta Samhita has included Swarna under ParthivaDravya and has mentioned the properties of Swarna as Tridosahara, Visapaham, Bruhaniya, Chakhsushya, Rasayana, Hrudya, Madhura Rasa and SheetaVirya. In Astangahrudaya, Swarna is included under Madhuragana. In most of references ‘Swarna’ was attributed to superlative qualities. Earning of gold and possessing it was considered auspicious. Swarna was used as currency as rewards by Royal families. Gold vessels were used for keeping ‘Soma.’

Gold has been used for its therapeutic properties both in Western and eastern world. Madihassanin 1985 investigated the use of gold in East. It is documented that the Chinese used red colloidal gold as the alchemical drug of Vitality and longevity. The word alchemy derives from Chinese words: Kim- gold and Yeh Juice. Kim-yeh (Golden juice) entered the Arabic language as Kimiyah, and in Arabic it was known as alkimiya now we know it as alchemy. The process of making red colloidal gold continued in India. SwarnaBhasma (Red gold) is prescribed by Ayurvedic physicians for rejuvenation and revitalization during old age and also for treatment of various ailments.

**Synonyms:** Swarna, Hiranya, Hema, Hataka, Tapaniya, Kaladhruta, Bhrama, Kanchana, Carmaker, Jatarupa.

**Utpatti:** According to ancient Ayurvedic texts Swarna is byproduct of Virya (Semen) of Agni (God of Fire).
Types of Swarna (gold): The 5 types of Swarna mentioned in the classics are:

1. Prakruta (Natural gold)
2. Sahaja (Native gold)
3. Agnisabhuta (Swarna prepared with the help of fire)
4. Khanija (Gold from the ores)
5. Rasaviddha (Swarna prepared by alchemist)

Prashastha/Grahya Swarna Laxan (Acceptable gold for medicine preparation)

Daheraktam - The Swarna (gold) which becomes red after heating

Seetamchhede - Shines after cutting,

Nikashekumkuma Prabham - Leaves reddish yellow colour (like Keshar) after rubbing on touch stone,

Guru - Heavy

Snigdha - Shiny

Mrudhu - Soft to touch

Swaccha - Clean/clear look

Nirdalam - Without any layers

Rakta Peetakam - Reddish yellow in colour

Shodasha Varnadyam - Swarna which has 16 colors is best for Rasa - Rasayanaand

Prashastha/Grahya (Acceptable) Swarna for preparation of medicines.

Aprashastha/Agrahya Swarna Laxana (Non Acceptable gold for medicine preparation): The Swarna (gold) which becomes white after heating, does not shine after cutting, leaves black or white colour after rubbing on touch stone, which is light,
non lusturous, hard to touch, of unpleasant appearance and has layers is Aprashastha (Non Acceptable) Swarna for preparation of medicines and for Rasa- Rasayana (for Rejuvenation).

**SwarnaShodhana:** The Swarna having above said acceptable characters should be selected for the preparation of Swarnabhasma. The general preparation of SwarnaBhasmainvolves the two processes i.e. Shodhana and marana.

Gold is believed to be the purest amongst all metals. It is extensively used in the Ayurveda as a medicine therefore; many authors do not feel the need for purification. However, gold which has Hinavarna (below the purity of 24 carat), needs Shodhana. According to Rasendra Sara Sangraha\(^ {21}\) and Ayurveda Prakash,\(^ {22}\) impure gold is likely to cause untoward effects. Impuregold when used internally may lead to loss of Saukhyya, Virya, and Balahani and also leads to diseases.

SwarnaShodhana is done by two methods

1. **Samanya** – This method of Shodhana is common for all dhatu.
2. **Vishesha** – This method is specific for specific dhatu or Swarna

**Samanya Shodhana:**\(^ {23}\) GrahyaSwarnaKantakavedhi (through which a thorn can be pierced) patra are prepared, heated over fire till the patra becomes red hot and is dipped in tilalata (Sesamumindicum). The procedure is repeated seven times by changing the Drava Dravya each time. Swarnapatra is processed in the same manner with Takra (buttermilk), Gomutra (cow’s urine), and the decoction of kulathya (Dolichosbiflorus), radish(Raphanussativus) andkanji (sour gruel processed from rice
[Oryza sativa}). Finally, the patra is dried by heating. Care must be excercised to retain the weight of Swarna.

**Vishesha Shodhana:**

KantakavedhiSwarnapatra-10 gm
Valmikamrittika- 40 gm
Ishtika churna-40gm
Grihadhuma -40 gm
Gairika - 40 gm
Saindhavalavana -40gm
Kanji - 200gm

All the ingredients are grouded together to make a fine paste. The paste is applied to kantakavedhiSwarnapatra and dried. The dried Swarnapatra is placed in sharavasamputa and subjected to kukkanuta puta. After swangasheeta the patra is rubbed with a clean cloth and used in further part of the procedure. By this method of shodhana, Swarna becomes more shiny and soft.

**Marana:** Different methods are available for Swarna Marana. One amongstthose procedures is explained below. A measurement of 15grams each of manashila (Arsenic disulfide), Realger (As$_2$S2), and red lead (Pb3O4) is taken in an earthenmortor and mixed thoroughly with 30 milli litres of Arkaksheera (latex of *Calotropis gigantea*). This mixture is thoroughly triturated to obtain a soft paste and then dried under sunlight. This process of triturating and drying in sunlight is repeated for 7 to 14 times using fresh Arka ksheera (latex of *Calotropis gigantea*), and the final product (~200gms) is obtained. The
above product (~10gms) is poured into liquefied metallic gold (10gms) in a closed earthen pot and the mixture is heated above 1000 C. The content is gently stirred and the heating is continued until the mass disintegrates and a homogenous red-brown powder is obtained. The ingredients of Swarna Bhasmaprepared by Ayurvedic Works, Kolkata, has been reported. The gold content was reported to be 96.76%. It also contained trace quantities of copper and iron.

The Organoleptic Characteristics of Swarna Bhasma

Colour – Champak Pushpa Varna (dark brown)
Odour -Nirgandha (no smell)
Touch- Slakshna (fine),
Taste -Nirgandha (tasteless).

Dosage – SwarnaBhasmamatra 1/8 to ¼ Ratti (15-30 mg)

Analysis of Swarna Bhasma: 28

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>SwarnaDhatu (Gold Metallic)</td>
<td>96.76%</td>
</tr>
<tr>
<td>Valuka (Silica – Si O₂)</td>
<td>1.14%</td>
</tr>
<tr>
<td>Loha (Iron- Fe₂O₃)</td>
<td>0.14%</td>
</tr>
<tr>
<td>Sudha (Lime – CaO)</td>
<td>0.55%</td>
</tr>
<tr>
<td>Tamra (Copper-Cu)</td>
<td>Traces</td>
</tr>
<tr>
<td>Mangnesia (M)</td>
<td>Traces</td>
</tr>
<tr>
<td>Phosphates (P₂O₅)</td>
<td>0.78%</td>
</tr>
<tr>
<td>Potasha (K₂O)</td>
<td>0.16%</td>
</tr>
<tr>
<td>Sodium Chloride (NaCl)</td>
<td>0.078%</td>
</tr>
<tr>
<td>Sulphate (SO₃)</td>
<td>0.15%</td>
</tr>
<tr>
<td>Moisture</td>
<td>0.24%</td>
</tr>
</tbody>
</table>

Indications: Swarna Bhasma is Madhura, Kashaya and tikta in Rasa and Madhura in Vipaka. Sheeta snigdha, guru, Vrushya, Brimhana, Medhya, Rasayana, Vajikarana, Ayushya, SantatiPradha, Pumsavanopayogi. SwarnaBhasmais also used in the treatment
of diseases such as anemia, dyspepsia, epilepsy, neurasthenia, loss of memory, bronchitis, asthma, tuberculosis, leucoderma, and rheumatoid arthritis.\textsuperscript{29, 30} The recommended dose is 1/4\textsuperscript{th} to ½ Ratti.

### Table 1

**Few Swarna Bhasma Containing Compound Formulations and their different indications**

<table>
<thead>
<tr>
<th>Name of the Formulation</th>
<th>Reference</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agnimukho Rasa (Tritiya)</td>
<td>Rasa Ratnakara, ShoolaRogadhikara</td>
<td>Gulma&amp;ShoolaRoga</td>
</tr>
<tr>
<td>Atisarantako Rasa</td>
<td>Rasayana Sara, AtisaraAdhikara</td>
<td>Atisara&amp;Pravahika</td>
</tr>
<tr>
<td>Amara SundariGuti (Pratham, Trithiya)</td>
<td>RasendraMangala</td>
<td>Jara&amp;MruthyuNashaka</td>
</tr>
<tr>
<td>AmrutaPrasha</td>
<td>Rasa yoga Sagara Page 80, Vol-1 Edition -2004</td>
<td>Ayu, Bala and AngapushtiVardhaka</td>
</tr>
<tr>
<td>Arkaanaleshvara Rasa</td>
<td>Yoga Manjari</td>
<td>ViryaVruddhikara</td>
</tr>
<tr>
<td>AshtaMurthi Rasa (Dwitiya &amp; Triphiya)</td>
<td>Rasa Chandamsu, Jwaradhikara</td>
<td>Kshaya, Pandu Roga, &amp;VishamaJwara</td>
</tr>
<tr>
<td>Ananda Bhairavo Rasa (Panchama)</td>
<td>Rasa Chintamani</td>
<td>DeergaKalinaPramehaNashaka</td>
</tr>
<tr>
<td>IndukalaVati</td>
<td>BhaishajyaRatnavaliMasurikaRog hadikara</td>
<td>MasurikaRaktagataJwara, Vruna</td>
</tr>
<tr>
<td>InduVati</td>
<td>BhaishajyaRatnavali Karna Rogadhikara</td>
<td>Karna Roga, VataRoga, Prameha</td>
</tr>
<tr>
<td>UnmadaGajankusha Rasa (Dwitiya)</td>
<td>RasayogaSagara Page 178 Unmadahara</td>
<td>Unmada, Rasayana</td>
</tr>
<tr>
<td>Unmade hara</td>
<td>Rasayana Sara Unmada</td>
<td>Unmada, Apasmara</td>
</tr>
<tr>
<td>Name</td>
<td>Source</td>
<td>Effects</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>------------------------------------------------------------------------</td>
<td>---------------------------------------------------</td>
</tr>
<tr>
<td>Kanaka Sankasmsho Rasa</td>
<td>Rasa Kamdenu, Kushta</td>
<td>Visphota, KushtaNashaka</td>
</tr>
<tr>
<td>Kanaka Sundaro Rasa (Trithiya, Chturtha,</td>
<td>Rasa RatnaSamuchyaya &amp; RasayogaSagara, Page 204-210</td>
<td>GudaRoga, Rasayana, Kshaya etc</td>
</tr>
<tr>
<td></td>
<td>Arshodhikara, Visarpaadhikara Vol-1, Edition -2004</td>
<td></td>
</tr>
<tr>
<td>Kanakambudo Rasa</td>
<td>RasayogaSagara, Page 211</td>
<td>Rajayakshma</td>
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<tr>
<td>Kandarpo Rasa</td>
<td>BhaishajyaRatnavaliPramehaRoghikara</td>
<td>Prameha</td>
</tr>
<tr>
<td>Kandarpa Sundaro Rasa</td>
<td>RasaprapakashaSudhkara, Vajkarana</td>
<td>Vajkarana</td>
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<tr>
<td>Kaphakutaro Rasa</td>
<td>Rasa Chandamshu, KaphaPrakarana</td>
<td>KaphaNashaka</td>
</tr>
<tr>
<td>Kalpataru Rasa</td>
<td>Yoga Manjari, Jwara</td>
<td>SarvaJwara</td>
</tr>
<tr>
<td>Trisankata Rasa</td>
<td>Rasa Kamadenu, Pandu Rogadhikara</td>
<td>Pandu Roga</td>
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<tr>
<td>Trilokya Chintamani Rasa</td>
<td>BhaishajyaRatnavali, KshyaRogadhikara</td>
<td>Amavata, VataRoga, Shwasa, Kasa, Kshaya, Kushta, Atisara etc</td>
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<tr>
<td>Dinajwaraprashamani Vati</td>
<td>Rasakamadenu, Jwara</td>
<td>Jwara</td>
</tr>
<tr>
<td>Divya Khechari Vati</td>
<td>Rasa yoga Sagara, page 647 Pratham Khanda</td>
<td>Rasayana</td>
</tr>
<tr>
<td>Dhanvanatari Vati</td>
<td>VaidhyakaChintamani, Sannipata</td>
<td>All Sannipata Roga Nashaka</td>
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<tr>
<td>Nagavallabha Rasa</td>
<td>Yoga Ratnakara, Kasa Shwasa</td>
<td>Kasa, Shwasa, galaganda, Gandamala, Timira etc</td>
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<tr>
<td>Naarimatta gajankusha Rasa</td>
<td>Vriddha Yoga Tarangini, Rasayana, Vajikarana</td>
<td>Rasayana, Vajikarana</td>
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<tr>
<td>Swarna Parpati</td>
<td>BhaishajyaRatnavali, Sangrahani</td>
<td>Sangrahani, Rajayakshma, Pandu etc</td>
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<tr>
<td>Vijaya Parpati</td>
<td>BhaishajyaRatnavali, Sangrahani</td>
<td>Atisara, Arsha, Sangrahani, Rajayakshma, Pandu etc</td>
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<tr>
<td>Makara Dwaja</td>
<td>Rasendra Sara Sangrahana</td>
<td>Rasayana Vajikarana</td>
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Review of Literature

<table>
<thead>
<tr>
<th></th>
<th>RasayanaVajikarana</th>
<th>vrushya, Kanti, MedhaVardhaka</th>
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<tr>
<td>Siddha Makara Dwaja</td>
<td>Rasa Tarangini 6</td>
<td>Vajikarana, vrushya</td>
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<tr>
<td>HemagarbhaPottali Rasa</td>
<td>Rasamrita, RasayanaVajikaranaAdhikara</td>
<td>RasayanaVajikarana</td>
</tr>
</tbody>
</table>

Researches Related to Swarna Bhasma: Gold and its compound formulations, as well as their potential therapeutic applications has been reviewed periodically over the years. At present, the most active area in developing gold based preparations is to investigate its antitumouractivity. Thus gold compounds are being actively studied and have also been investigated for anti-HIV properties as anti-malarial agents and even for the treatment of bronchial asthma. Swarna Bhasmaprepared by following the procedure as per RasaratnaSamuchchya which resulted in following physicochemical characteristics. Atomic absorption spectrometer (ASS) was used to study the contents of gold and other metals. Where the gold content formed to be 20.3%. Some other important metals that were present were cobalt, copper, iron, magnesium, lead, nickel and zinc.

Gold content in different Swarna Bhasma preparations: Vijay Yadav et al (2012) in their study reported that there is a difference in gold content in different Swarna Bhasma samples, which is statistically significant. They procured Swarna Bhasma form different sellers and carried out its physiochemical characterization. As control they selected Standard Gold nanoparticles and morphological details of the samples were studied with the help of SEM. Swarna Bhasma particle size ranged from 3μm-135μm. Energy Dispersive Spectroscopy (EDS) analysis of Swarna Bhasma showed Au-content in one sample and 92.19 % Au in another sample. Standard gold nanoparticles showed 100 % Gold content. Swarna Bhasma has various therapeutic applications. Particle size and concentration of Gold have significant effect on its absorption, assimilation and flow in the body hence variable Swarna Bhasma samples might have variable effect on the body.
Utility of Swarna Bhasmikaran process: Swarna (Gold) is one of the most non-reactive metals of all times, as known to mankind. To make Swarna therapeutically useful, two important procedures need to be done. First is reduction in particle size and converting it into suitable for absorption.

Physico-Chemical Characterization of Swarna Bhasma: Hillyer J F et.al(2001)in their study evaluated Swarna Bhasma with the help of Atomic Absorption Spectrometer, Infrared Spectroscopy, Transmission Electron Microscope, Atomic Force Microscope and X-ray Diffraction method. Atomic absorption spectroscopy revealed that Swarna Bhasma contains 92 % gold. Cold vapor atomic absorption spectroscopy demonstrated absence of organic compounds and mercury can be considered as a proper incineration. The study has shown Swarna Bhasma contains globular gold particle of 56-57 nm. By using instrumental neutron activation and electron microscopy analysis the gastrointestinal absorption and distribution of metallic colloidal gold particles having 58nm, 28nm, 10 nm and 4nm diameter following oral administration to mice. Study showed that particle uptake occur in small intestine by persorption through single degrading enterocytes from a villus. Basically Swarna Bhasma constituted globular gold particle of 56-57 nm and thus these particles through gastrointestinal uptake would reach the target site of action through blood.

Analgesic Activity: Bajaj and Vohora (1998) have studied Analgesic activity of Swarna Bhasma involving mice. A Unani calcined gold preparation, Kushta Tilan Kalan (KTK), was used along with the gold drug auranofin. Swarna Bhasma at 12 to 50 mg/kg body weight by oral route showed analgesic activity. Whereas the analgesic effect of Swarna Bhasma could be blocked by the treatment of nolaxone, such antagonism was not possible in the case of auranofin.
**Analgesic Activity:** \(^{61}\) In an experimental study, by using four types of noxious stimuli, *Swarna Bhasma* was investigated for analgesic effects in rats and mice. It was observed that, the test drugs *Swarna Bahasma* at a dose of 25-50 mg/kg, p.o exhibited analgesic activity against chemical, electrical, Thermal and mechanical test.

**Immune Response:** \(^{62, 63}\) Swarna bhasma-treated in mice in the range of 12.5 to 50 mg/kg body weight showed modification in the specific and nonspecific immune response. This may be helpful to fight against infections.

**Antioxidant Activity:** \(^{64}\) The antioxidant and restorative effects of *Swarna Bhasma* in rats have recently been demonstrated. The study was done by giving phenobarbitone anesthesia, where ischemia induced by bilateral carotid. A number of antioxidant enzymes and lipid peroxidation were studied. *Swarna Bhasma* (25 mg/kg) had a significantly restoring antioxidant activity in ischemic animals. Biochemical findings correlate the histological examination of the brain. Several studies on *Swarna Bhasma* have shown free radical scavenging and antioxidant effects by measuring antioxidant enzymes superoxide dismutase (SOD) and catalase after oxidative insult with acetic acid in swarna bhasma-treated mice as well as control serum and liver homogenate of mice. *Swarna Bhasma* induced had no toxic effects. This was assessed by liver function test and histological investigations.

**Suvarna Bindu Prashan Toxicity Study in Albino Rats:** \(^{65}\) To evaluate the toxicity of Suvarna Bindu Prashan in experimental animals for 90 days duration study. Preparation of Swarna Bhasma and Suvarna Bindu Prashan and Physio-Chemical analysis of raw
Review of Literature

materials and prepared sample were done with the Hypothesis: Use of Suvarna Prashan has been recommended for neonate for long term and claimed as a wide range of therapeutic efficacy in classics. Its modified form Suvarna Bindu Prashan may have some unknown toxicity profile. Equal quantities of ghee and honey may have influence factor in role of toxicity. Methodology: For the preparation of Swarna Bhasma and Suvarna Bindu Prashan raw materials were collected as per grahya lakashan. For the procedure of Swarna Bhasma preparation, samanya shodhana, vishesh shodhana Suvarna marana was done. Materials were analyzed to physio-chemical characteristic in AYUSH certified laboratory, KLEU, Shri BMK Ayurved Mahavidyalaya, Belgaum. Chronic Toxicity Study was carried out for 90 days as per OECD guidelines where in five group of animals consisting of six animals in each group were meticulously studied. Study design was sanctioned for ethical clearance by IAEC (BMK/IAEC/Res-03/2009). Results of Physico-Chemical analysis of raw material were found to conform to standard values of API. In XRD, analysis of Swarna Bhasma showed presence of Au. SEM analysis indicated particle sizes in range 40-100nm. EDXA study showed element of Au 94.02% in Suvarna bhasma. No mortality and abnormal behavioral changes were found in albino rats in chronic toxicity level study. Maximum increase in weight was observed in SBP group (116.42%) compared to control group (92.77%) and other groups. No remarkable pathological changes were observed in organs of both control and SBP group. Few hematological and biochemical parameters changes were noticed in SBP group. But values were found within the normal range or nearer to normal range. Histo-pathological examination of organs showed normal cytoachitexture in SBP group. The mild to
Review of Literature

moderate triditis was observed in the liver of test group ghrita, but no sign of severe toxicity were seen in other test group. No signs of toxicity were observed in any group. Histopathology showed mild congestion in most of the organ tissues. This is considered as normal. On the basis of the results of present study the researcher has concluded that Suvarna Bindu Prashan did not produce chronic toxicity. But few changes were noticed at level of hematological and biochemical parameters. Hence it was suggested that further study is needed to analyse safety parameters in higher species having large sample size.

**Immunomodulatory Study of Suvarna Bindu Prashan in Albino Rats:** To study immunomodulatory effect of Swarna Bindu Prashan in Albino Rats. Physico-Chemical Analysis of Swarna Bhasma, Ghrita and Madhu with the hypothesis that there are various formulations administered to infants, to prevent various diseases and maintain positive health in Ayurveda. Suvarna Bindu Prashan is a modified form of one of these formulations practiced in our institute which contains Swarna Bhasma, Ghrita and Madhu (4mg + 0.2ml and 0.2ml respectively). All the findings of this are subjective, exact effect of the formulation on the children is not studied, previous observation shows children receiving ‘Swarna Bindu Prashan’ suffer less and recover faster from illness. This gives the hypothesis that ‘Swarna Bindu Prashan’ has immunopotent effect, but the exact mode of action immunity is not known. Hence this study highlights the mode of action of ‘Swarna Bindu Prashan’. For the study Swarna Bhasma was prepared by classical reference at Ayurveda Rasayani Pharmacy, Pune by referring to classical literature. Swarna Bhasma, Ghrita and Madhu were subjected to qualitative and quantities analysis. Immunomodulatory study was done by taking parameter phagocytic activity by
calculating carbon clearance test in male wistar albino rats. For this 24 animal were divided into four groups; Group I (Control), Group II (Madhu+Ghrita), Group III (Suvarna Bindu Prashan) daily for 10 days and Group IV (Suvarna Bindu Prashan once in 15 day for 3 month). The Swarna Bhasma was brick red colour and passed all the classical bhasma parikshas(tests). XRD Analysis showed peaks at 2θ value of bhasma were identical with standard gold metal (Au). SEM report showed most of the particles in nano range from 40 nm to 100 nm • AAS report showed 94.02% Au with other trace element. Ghrita showed LOD 0.3%, Refractive Index 1.46, Sp.Gr. 0.8987, Acid Value 1.683, Saponification Value 219.55, Iodine Value 54.95 and Negative Rancidity. Result of Madhu sample A passed Fieche’s test (cherry red colour indicates addition of Artificial Invert Sugar in all other samples of Honey). Experimental study has showed that Group III shows increased phagocytic index over Group I, Group II and Group IV and phagocytic index decrease in Group IV than Group III. With these results the researcher concluded that immunomodulatory study shows significant result for Group III over all the Groups and Group IV shows significant result over Groups I and II, but nonsignificant result over Group III (p<0.0001). This concludes the Swarna Bindu Prashan has immunomodulatory effect on albino rat.

**Immunomodulatory Activity:** In Kashyap Samhita, while describing the benefits of Swarna Lehana, Acharya Kashyapopines that, a child fed with gold for a month becomes immune to diseases. This classical description implicates that ingestion of Swarna modifies immune mechanism, so that morbidity is reduced. Now, it is matter of debate regarding the scope of Swarna Bhasmain modulating the immune mechanisms of the
newborn, so that, the child will not get any sort of disease. Research papers on the effect of Swarna Bhasma on immunity are very few. In an experimental study, Bajaj et al (2001) evaluated the efficacy of Swarna Bhasma on non-specific immunity in mice. Male mice were administered with the incremental doses of Swarna Bhasma orally for 10 days. It was observed that, Swarna Bhasma significantly (p<0.001) increased macrophages counts of peritonea and stimulated phagocytic index. This demonstrates the immunostimulant activity of traditional Ayurvedic formulation ‘Swarna Bhasma’ on macrophage functions. A gold formulation, Kustha tila kalan used in Unani-tibb was evaluated for immune modulatory activity in male mice. The effects on cell mediated as well as humoral immunity were evaluated. Kustha Tila Kalan was orally administered to animals at dosage of 6.25mg, 12.5mg, 25mg and 50 mg/kg body weight for 10 days. Immunity was assessed by measuring cell mediated delayed type of hypersensitivity response was evaluated. Kustha Tila Kalanaugmented both the immune responses at dose level of 6.25mg, 12.5mg and 25 mg/kg. The maximum activities were observed at a dose of 25 mg/kg.

**The Effects of the Mode of Delivery on Oxidative-Antioxidative Balance:** Mitra et al (2002) evaluated the free-radical scavenging activity of Swarna Bhasma on experimental animal. It was observed that chronic administration of Swarna Bhasma treated animals showed significantly increase in SOD and catalase activity. These two enzymes are responsible for reduction in free radical concentration in the body. Antioxidant/restorative effects of Swarna Bhasma on focal models and global of ischemia (stroke) are also reported. Acharya Kashyap explains that, babies born to Dushta
Prajatamothers should be administered with Lehana (Swarna Lehana). Dushta Prajataliterally means women with the past bad obstetrical history.

Now, it is a matter of logical reasoning why ancient scholars suggested giving Lehana to babies born to Dushta Prajatamothers. With medical knowledge ever expanding and developing, in the recent times, scheduled caesarean section is performed on Dushta Prajatamothers in order to reduce morbidity and mortality. Investigational studies on differences between babies born to normal mother and Dushta Prajata mothers is a matter of interest.

A very recent study by Mutlu B et.al (2011) evaluated the effects of the mode of delivery on oxidative and antioxidative balance of mothers and infants. It was observed in scheduled caesarean section group both the mothers and neonates were exposed to higher oxidative stress as compared to those in normal vaginal deliveries group. Same study reported that the antioxidant effect in babies are insufficient to cope with this stress during caesarean section.

**Swarna Bhasma in Restraint Induced Stress at Different Time Points:** Shah et al (2005) investigated the therapeutic potential of Swarna Bhasmain stress induced at different time points of 01 hour, 02 hours and 04 hours using on rat model. Rats were pretreated with *Swarna Bhasma* in a dose of 25 mg/kg orally for 10 days prior to induction of stress. Brain serotonin, catecholamine and plasma corticosterone levels were determined following 01, 02 and 04 hours stress, using HPLC and also plasma corticosterone using luminescence spectrophotometry. It was observed that Swarna
Bhasma restored stress induced elevation in levels of brain norepinephrine, dopamine, epinephrine, 5 HT and plasma corticosterone to near normal levels.

**Toxicity Studies:** In an experimental model, it was observed that, acute oral administration of Swarna Bhasma showed no mortality in mice (upto 1 ml/20 g body weight of Swarna Bhasma suspension containing 01 mg of drug). Moreover, chronic administration of Swarna Bhasma also showed no toxicity as judged by Serum Creatinine, SGOT, SGPT, and serum urea level and histological studies.

**Anti-Inflammatory and Immunomodulatory:** A gold preparation used in Unani-Tibb in the name of Kushta Tila Kalan (KTK) possesses anti-infective and rejuvenating properties. Its immunomodulatory activity was evaluated in male mice. KTK was orally administered to animals at dosage of 6.25, 12.5, 25 and 50 mg/kg body weight for 10 days. Besides general immuno-pathological parameters, cell-mediated immunity was evaluated by measuring delayed hypersensitivity response (DTH) while humoral immunity was assessed using plaque forming cell (PFC) assay. KTK augmented both the immune responses at dose levels of 6.25 mg, 12.5 mg and 25 mg/kg. The optimum activities were recorded at 25 mg/kg. Higher dose of 50 mg/kg showed suppressive effects on immune functions. In the pharmacological study, Madhu-Ghrita combination was found to have very good immuno-potentiating activity in terms of both humoral anti-body formation and cell mediated immunity. However Madhu-Ghrita-Swarna-Vacha combination only had the effect of humoral anti-body formation. In the clinical study, Madhu-Ghrita and Madhu-Ghrita-Swarna-Vacha, both acts as equivalent immunomodulators for Neonates. They have definite action on immunological system as evidenced by triggering the response of immunological system by a rise in the total proteins and serum IgG levels as compared to its fall in control group.

**Clinical Studies**

**Treatment of Asthma:** The efficacy of parenteral gold therapy was evaluated in patients with steroid dependent asthma. Five of eight patients improved in terms of
reduced steroid requirement while they were maintaining or improving lung function. Two patients developed proteinuria and protein levels reduced with decreased levels of gold. Chrysotherapy appear to have a corticosteroid-sparing effect in some patients. Hence its role in the management of severe refractory asthma should be further assessed.

**Gold Containing Ayurvedic Preparation Toxicity Study:**\(^{73}\) Gold containing Ayurvedic preparation, Swarna Vasanta Malati, was studied for safety. The bhasma was given to 20 male persons at a dose of 100 mg twice a day for 40 days under supervision by Ayurvedic physicians. The total cumulative intake of 160 mg of gold at the rate of 4 mg/day did not show any toxic effect on human body, as evidenced by clinical examination, unaltered body weight, normal urine.

**Treatment of Arthritis:**\(^{74,75}\) A comparative clinical study was carried out by giving cyclosporin and parenteral gold for three years and it showed similar changes in patients with prediagnosed Rheumatoid Arthritis. Abnormal renal function and raised blood pressure were often seen in the cyclosporine treated patients as compared to gold treated patients\(^{42}\). Colloidal clanging gold (Average particle size 27 nm) is far more potent and effective anti arthritic agent in rats than sodium aurothiomalate used to treat Rheumatoid Arthritis.

**Use of Gold Nano Particles in Leukemia:**\(^{76}\) Priyabrata Mukherjee et.al has studied potential therapeutic utility in the form of Gold Nanoparticles in B-chronic lymphocytic leukemia. B-chronic lymphocytic leukemia (CLL) is an incurable disease predominantly characterized by apoptosis resistance. Induction of more apoptosis was seen when along
with CLL B –cells, an anti-VEGF antibody was cultured. To increase the efficacy of CLL therapy they used gold nanoparticles (GNP). Gold nanoparticles were used based on their biocompatibility, surface area, easy to characterize and function. VEGF antibody (AbVF) was fixed to the gold nanoparticles and determined their ability to kill CLL B cells. Characterisation of Gold nanoparticles and their Nanoconjugates was done by using UV-Visible spectroscopy (UV-Vis), Transmission Electron etc instruments. All the patient samples studied (N = 7) with a dose dependent apoptosis of CLL B cells. The induction of apoptosis was more with gold-AbVF. The gold-AbVF treated cells showed significant decrease in anti-apoptotic proteins. Gold-AbVF treated and GNP treated cells entered internally in early, late endosomes and many vesicular bodies. Non-coated gold nanoparticles were also able to induce minimum level of apoptosis in CLL B cells.

Clinically Evaluate the Effect of Suvarna Bindu Prashan on Immunity and Intelligence of Children:  

Suvarna prashan is one of the formulations explained in age old Ayurvedic classical text Kashyap Samhita for promoting mental and physical health of a child. This formulation is very widely used now a day as a memory and immune booster for children. Suvarna Prashan is already in practice in many Ayurvedic institutions, hospitals and clinics throughout India. This procedure has achieved a great success in all centers. But there is very few systematic documented study which can be used to evaluate the efficacy of the formulation. The study aimed at clinical evaluation of the effect of Suvarna Bindu Prashan on Immunity and Intelligence of Children. Methodology: The Swarna Bhasma
was prepared in Ayurved Rasayani Pharmacy, Pune. Madhu and Ghrita were collected from KLE Ayurveda Pharmacy, Belgaum. Suvarna Bindu Prashan was prepared in KLE Ayurved Pharmacy, Belgaum. It contains Suvarna Bhasma, Ghrita and Madhu (100 mg Swarna Bhasma + six (6) ml Ghrita + six (6) ml Madhu). Analytical study qualitative and quantitative analysis of Madhu and Ghrita were carried in Central Research Laboratory of KLEU’s BMK Ayurvedic College, Belgaum. Analytical data of Swarna Bhasma were collected from Ayurveda Rasayani, Pune. Parents willing to administer Suvarna Bindu Prashan to their children were screened in Suvarna Bindu Prashan camp of K.L.E.Ayurvedic hospital, Belgaum. Twenty apparently healthy male and female children within the age group of three to four years whose parents were ready to give informed consent were selected and divided into two groups each equally. Subjects in Group A received Suvarna Bindu Prashan whereas Group B (Control group) did not receive any treatment. Both the groups were observed for six months. Data of height, weight, quality of life, event of illness were collected every month. IQ and Immunoglobulin’s (IgG, IgM, and IgA) levels were assessed before and after treatment. The study was designed as open label, parallel, prospective and observational study. Results of children in Suvana Bindu Prashan group showed significant reduction in temperamental behaviour, mood-swings and scores of event of illness. However, sleeping habit was not significant. IQ percentage was statistically significant. With above findings researcher has concluded that SBP is helpful in improving immunity and intelligence.
Swarna Bhasma in cancer: Das, et al.: Role of Swarna Bhasma in cancer (2013) has published an article in AYU Journal entitled Swarna Bhasma in Cancer: A Prospective Clinical Study. The study was conducted in the Calcutta Gastroenterology Research Centre (CGRI). The study conducted about 5 years. Institutional Ethics Committee (IEC) approval was taken and study was conducted to determine outcome of SB on solid malignancies. Out of the total of 43 patients included in the study who received Swarna Bhasma for 1 year, 17 patients showed response. The response was best in rectal cancer group 70% (7/10). With the treatment 41% patients lived for 1 year. After 5 years this came down to 15.38%.

Blood Compatibility Studies of Swarna Bhasma: Swarna Bhasma preparation did not induce any blood cell aggregation or any Protien adsorption. Activation potential of these towards complement system or platelet was negligible. Study has shown that Swarna Bhasma has ability to open up the tight junction and is taken up by small intestine when administered orally to reach the affected site via blood and release Au ions in sustained manner. The gold particles were non cytotoxic.

A 24-karat gold key to unlock the immune system: Using nanoparticles made of pure gold, a new method of introducing chemical residue into the immune system, which triggers immune cells to help the body fight infection. The breakthrough could lead to understand viruses, bacteria and drug delivery. Because the gold flecks were very small to be identified by the immune system, the immune system responds when they are coated with different chemicals. The hydrophobic areas of the cell membrane become bare.
during cell demise. The immune system then realizes the damage and begins to alert neighboring cells.

**Traditional Use of Swarnamrita Prashana as a Preventive Measure:** \(^{81}\) 16 centres were selected in and around Hassan.

- Group 1 - Children aged between 0-16 were administered Swarnamrita Prashana on pushya nakshatra for 6 months.
- Group 2 - 30 children bet 3-8yrs participated in clinical study they were administered 1ml swarnamirta prashana every day for 30 days.

**Reproductive Health:** Since centuries gold is used for treating male infertility in Ayurveda. Keeping this in mind, semen analysis by atomic absorption spectrophotometry was carried out. The value of gold in semen was found to be in a range of 0.36 to 1.98 μg/ml\(^{82}\). The seminal work carried out on AuNP-DNA assemblies is very promising for biological applications such as labeling, detection and transfer of drugs, including genetic material.\(^{83}\) Recent study for blood compatibility study for Swarna Bhasma showed their ability to open tight junction integrity as per Caco-2 experiments. It has been demonstrated that gold nano-particle uptake occurs in small intestine through degrading enterocytes from a villus. GNP of size less than 58nm reaching various organs through blood show importance of its blood compatibility study\(^{84}\). A clinical study carried out on Chandrodaya Rasa; Swarna containing formulation on its efficacy on Shukrakshaya (oligospermia) showed statistical significant result in spermatogenesis. There was no significant improvement in the volume which denotes that drug improves the semen qualitatively rather than quantitatively. No significant side effects were found during
the study. In study carried out of Swarna Bhasma on patients of ShukraDushti for four months showed significant results. It is found that RLP motility and SLP motility was significantly increased. Immotile spermatozoa count was significantly decreased. Volume of semen was significantly increased. Liquefaction time, viscosity, pH, debris material and amorphous matters of semen were significantly decreased. Viability and total abnormal forms of spermatozoa were reduced significantly. Sperm function test, hypoosmotic swelling test recorded significant increase in all the four months with peak after four months. 10 patients of KshinaShukra (Oligozoospermia) were treated with Swarna Bhasma (10 mg twice daily in capsule form with milk) for period of 30 days. The results showed insignificant increase in sperm count along with RLP motility. Regarding the efficacy of Swarna Bhasma on sexual health, significant amelioration was attained in the erection, ejaculation and orgasm. Godatwar PR conducted a clinical (4 mg twice daily for 1 month to 13 patients) and experimental study on the role of Swarna Bhasma in normozoospermia. Highly significant increase in bodyweight was observed. Significant improvement in the spermatozoa concentration, RLP and SLP motility were observed with corresponding decrease in NP and immotile spermatozoa. The experimental study proved that concentration of spermatozoa in the cauda epididymus of rat and RLP motility increased significantly. The area of interstitial tissue of testis was significantly increased. The diameters of the seminiferous tubules per field in rat testis were also increased. Swarna Bhasma has better proliferative activity on spermatogenesis as well as in enhancing the steroidogenic activity. Swarna Bhasma seems to be active at the level of mitochondria. Hence, this drug may be useful in treating male
infertility due to asthenozoospermia. It may also be recommended for treatment of early ejaculation\textsuperscript{90}. 0.5 mg gold chloride treatment for 26 days caused a significant increase in plasma T along with stimulation of testicular $\Delta_5 -3\beta$-HSD (Hydroxysteroid dehydrogenase) and 17 $\beta$-HSD activities. Gametogenic activity exhibited a significant increase in the number of step 7 spermatids at stage VII of seminiferous cycle when compared to control\textsuperscript{90}. Significant increase in ovarian and uterine weight $\Delta_5 -3\beta$-HSD (Hydroxysteroid dehydrogenase) activity and rise in serum estradiol level was observed after gold chloride (0.2 mg/kg body weight/day) subcutaneous administration in immature female albino rats. Histological study of ovary also showed Graffian follicle with ovum in rats proving stimulation of reproductive function.\textsuperscript{91}
Table 2
Biologically Active Gold Compounds in the Market\textsuperscript{92}

<table>
<thead>
<tr>
<th>No</th>
<th>Generic Name</th>
<th>Trade Name</th>
<th>Gold Concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Goldsodium thiomalate Myochristin</td>
<td>Myocrisin, Tauredon</td>
<td>50.5</td>
</tr>
<tr>
<td>2</td>
<td>Gold thioglucose</td>
<td>Solganal</td>
<td>50.5</td>
</tr>
<tr>
<td>3</td>
<td>Gold thioglycoanilid</td>
<td>Lauron</td>
<td>54.2</td>
</tr>
<tr>
<td>4</td>
<td>Goldsodium thiosulphate sanochrysine</td>
<td>Aurothion, Thiochrysine</td>
<td>40.2</td>
</tr>
<tr>
<td>5</td>
<td>Calcium aurothiothioglycolate</td>
<td>Myoral</td>
<td>64.1</td>
</tr>
<tr>
<td>6</td>
<td>Sodium2aurothiobenzidazole-4-carboxylalte</td>
<td>Triphal</td>
<td>47.8</td>
</tr>
<tr>
<td>7</td>
<td>Sodium auroallylthiourea-m-benzoate</td>
<td>Lapion</td>
<td>43.4</td>
</tr>
<tr>
<td>8</td>
<td>S-triethylphosphine gold 2,3,4,6-teta-O-acetyl-1thio-b-D-glycopyranoside</td>
<td>Auranofin</td>
<td>29.1</td>
</tr>
<tr>
<td>9</td>
<td>Chloro (triethylphosphine) gold</td>
<td>SKandF 36914</td>
<td>56.2</td>
</tr>
</tbody>
</table>

**Gold Modern Review:** Gold is a dense, soft, shiny, pliable and tensile metal. It is a chemical element with the symbol Au and atomic number 79. Gold is having shiny yellow colour, it is not affected by air and water. Chemically, gold is a conversion metal and component of group 11. Non-reactive chemical element under standard condition. It occurs often in the native free form, as grains in rocks, in the veins and in the alluvial deposits. Gold is unaffected by alkalies and acids, but it dissolves in aqua regia (nitro-
hydrochloric acid). Gold also dissolves in alkalines like cyanide, which have been used in mining. Mercury is capable of dissolving Gold, forming amalgam. Gold is insoluble in nitric acid.

**Etymology:** 93, 94 From the Old English or Old German gulth meaning bright and ghol meaning yellow or the Sanskrit word jval means to shine. Au is from the Latin: aurum, according to some sources meaning "shining dawn", 95 from Sabine ausum "lustroussunrise." In Latin also the meaning is same for aurum. 96, 97&98

**Characteristics:** 99. From 1 gram of gold 1 square meter sheet can be prepared.A transparent gold leaf can be prepared. The transmitted light looks greenish blue. Since, gold powerfully reflects yellow, red and infrared lights. 100 Gold readily creates alloys with many other metals. Alloys of Gold changes metallic properties of other metals. 101 Gold is a good conductor of heat and electricity and reflects infrared radition strongly. Gold not affected by air, moisture, most acids and alkalies. Therefore used to prepare coins and jewellery. Gold is almost insoluble, but can be dissolved in aqua regia. Pure gold is tasteless and odourless. 102

**Color:** 103 & 104 whereas most other pure metals are gray or silvery white, gold is yellow.

**A Brief History of Uses of Gold in Health:** 105 The earliest records of the use of gold for medicinal purpose trased from Alexandria, Egypt. More than 5,000 years ago, the Egyptians used to use gold for mind, body and spiritual cleansing. More than 4,500 years ago, the Egyptians used gold in dentistry. Its early use has been found by modern archaeologists. As an ideal material for dental work, approximately 13 tons of gold is
used each year for crowns, bridges, inlays and dentures. Alchemists used to use powdered gold into drinks.

**Food and drink:** Gold can be used in food and has the E number 175. Gold in the form of thin leaf or powder is used on and in some gourmet foods, notably sweets and drinks as decorative ingredient. Gold flake was used to prepare traditional German herbal liqueur. There were some expensive cocktails which contain gold leaf flakes. However, since metallic gold inert, it has no taste and it leaves the body unaltered.

**Toxicity:** Pure metallic (elemental) gold is non-toxic and is not irritating if swallowed. Gold leaf is used for food decoration. Metallic gold is also a component of the alcoholic drinks, Gold strike and Gold wasser. Metallic gold is approved as a food additive in the EU (E 175 in the Codex Alimentarius.) Although gold ion is toxic, but metallic gold is used as food additive due to its resistance to corrosion. Gold salts like gold chloride are toxic to the liver and kidneys. Gold contacts may cause allergies in women in few cases. Gold rarely produces allergies, in comparison with metals like nickel.
1.2.2 Kaseesa

The Names of Kaseesa in Different Languages are.

- English: Green vitriol, ferrous sulphate
- Arabi: Jaje Afasar
- Germany: Schwerelsaures Fisenxyduc
- French: Sulphate ferrent
- Latin: Ferri sulphos
- Hindi and Sindhi: Kaseesa
- Marathi and Konkani: Hira Kasa
- Gujarati: Hira Kaseesa
- Bengali: Hire Kasa
- Punjabi and Kashmiri: Sangai Sabja
- Tamil and Kannad: Annabhedi
- Parsi: Tritiyasaba
- Baluchi: Ladha

Vernacular Names in Sanskrit are as follows:

They can be classified into four captions:

1) According to Origin: Ayogandhamla, Pansuka, Dhatu Kaseesa
2) According to Characteristics: Kesharam, Pushpa Kaseesa, Valuka Kaseesa, Hira Kaseesa,
3) According to Properties: Danta Ranjakam, Kaseesa,
4) According to Action: Kesharanjakam, Dhatushekharam, Kansakam, Nainausadham, Chakshukam.

Sanskrit Synonyms with Meanings:

- Ayogandhamla: It contains iron and sulphuric acid.
- Kaseesa: It increases body luster.
- Kaseesa haka: It increases body luster.
- Kesharam: It is water soluble.
- Kesharanjakam: It is used in hair dyeing.
- Kansaka: It is used in Kapahaja Vyadhis like Shosa, Kasa etc.
- Danta Ranjakam: It discolours the teeth.
- Dhatu Shekharam: Kaseesa is given first preference amongst the other preparations in the disease anaemia.
- Hira Kaseesa: Due to its greenish colour, it is also named HiraKaseesa.
Review of Literature

Historical Review

Veda Kaala: No reference of Kaseesa is available in Vedas.

Samhita Kaala:

Charaka Samhita: There are 14 preparations of Kaseesa described in charaka Samhita. Kaseesa was used externally in the form of Lepa, Varti and Taila in the following conditions like Kustha, Visarpa, Vrana as lepa, Khalitya in the form of Manahashila Taila, Yonivyapada, as Yoni Varti.

Sushruta Samhita: There are 15 preparations of Kaseesa described in Sushruta Samhita in the form of Rasa Kriya in Vrana Pralepa in Pandukarma Lepa in Romasanjanana, Avachurnananain Galaganda and in Upadansha. Pratisarana in Alasa, Saireyakadi Taila form in Kesharanjana, Anjan in Praklinna Vartma, Sirotpata, Siraharsha, Arjuna, Puyalasa, Kaphaja Timira.

Ashtanga Hrudaya: In Ashtanaga Hrudaya Kaseesa, is mentioned externally and internally in the form of Churna, Lepa, Pichu and Taila. In Kumbha Kamala in the form of Churna + Milk, in Visarpa in the form of Lepa, in Twak Roga and Kustha in the form of Avachurnana, in Kilasa as lepa, in Puyalasa in the Anjana, in Krimigranthi in the form of Pratisarana, in Netra Kandu and Pakshamashata in the form of Anjana, in Apakva Talupaka in the form of Avachurnara, in Indralupta, Vranavasadana, Upadansha in the form of lepa. In Yonivyapadain the form of taila pichu and churna respectively.
**Rasa Kaala:** Rasashastra saw its development from 8th to 17th century, and this duration is believed as a golden period to Rasashastra, where researchers and devotees of Rasashastra probed deeper into the subject and developed remarkable literature for use. The sacrificing unselfish word of great Nagarjuna is a lesson and model to the Ayuverdic generation.

**Rasarnava (12th A. D.):** Rasarnava is a form of conversation between Parvati and Shiva. The author of which is not known. In this text the colour of Jwala of different Dhatu, Upadhatu, Rasa, Uparasa have been narrated. Here instead of ‘Adhyaya’ the word ‘Patala’ is used. There are 18 Patala in this book. Where the author has mentioned the properties, purification and varieties of Kaseesa, in a systemic manner.

**Rasaparaksha Sudhakara (13th A. D.) - (Yashodhara Bhatt):** The author has mentioned the properties, purification methods of Kaseesa. He has also mentioned properties of Rasakarpura.

**Rasendrasara Sangraha (13th A. D) (Gopalkrishna Bhatt):** This book is divided into two parts; the first part consists of a single chapter of description of Shodhana and Marana procedure, while the other part includes Yogas, several Rasa medicines and their usages. The Shodhana and Marana of Kaseesa is also found here.

**Rasaratna Samuchchaya (13th A. D) (Vagbhata):** This is also the best book amongst the Rasashastra literatures, where the author has mentioned Parada, Maharasa, Uparasa, Sadharana Rasa, Ratna, Uparatna, Lauha etc. systematically after compiling several books of Rasashastra. Also, the author has described the sources, varieties, properties, Shodhana, Marana and Satvapatana of Kaseesa in magnificently.

**Bhava Prakasha (17th A. D) (Bhava Mishra):** This book mentions the properties and uses of Kaseesa are mentioned in beautiful.
Rasakamdhenu (18th A.D) (Chudamani Mishra): There is a clear mention of the varieties, properties, Shodhana, Marana of Kaseesa by the author.

Rasatarangini (20th A.D) (Sadanand Sharma): This is one of the most efficient manuscripts in Rasashastra of present era. The author has mentioned thoroughly the synonyms, varieties, properties, Shodhana and Marana of Kaseesa.

Rasa Jala Nidhi (20th A.D) (Bhudev Mukharjee): The Satvapatana, Shodhana and Marana of Kaseesa have been satisfactorily narrated.

Rasamrita (20th A.D.) – Acharya Yadavji Trikamji: This is an excellent and main record of Rasashastra, where the author has described the properties, Shodhana and Marana of Kaseesa extraordinarily.

Commentary of Rasa Ratna Samuchchaya – Dr. D. A. Kulkarni: The above mentioned authors have mentioned the sources of Kaseesa, Shodhana, Marana, usages, dosages and several Yogas of Kaseesa systematically. Also they have dealt with the literature in modern and ancient line of approach. However, at I.P.G.T. and R.A., Jamnagar, a research work has been done on Shuddha Kaseesa Churna and Kaseesa Bhasma in Pandu and has proved its action.

Classification of Kaseesa: For convenience, Rasa Dravyas are classified into different Vargas i.e. Rasa, Maharasa, Uparasa, Sadharana Rasa and so on. It is very difficult to describe, on what basis this classification has been done by the commentator of Rasaratna Samuchchaya. Vaidya Dudhagaonkar provided his opinion that dravyas which are most useful in Parada Karma are taken in Maharasa and so on in descending order. Different authors have classified Kaseesa under different groups based upon their individual research. In Charaka Sutrasthana 1/71, Kaseesa has been described under Bhauma Gana,
while Acharya Sushruta has described it in Ushakadi Gana (Su. Su.38/37), whereas Acharya Vagbhatta has denoted it as Parthiva Dravya.

**Varga:** Uparasa - Rasarnava, Rasa Chintamani, Rasa Ratna Samuchchaya, Rasopanishada, Rasendra Sara Sangraha, Rasa, Chudamani, Rasa Hridaya Tantra, Ayurved Prakash. Upadhatu varga by Sharangadhara Samhita, Rasa Tarangini, Rasa Dhatu Prakasha. Dhatu Varga by Rasamrita (Lauha Varga)

**Description of Kaseesa:** There is no clear cut description in ancient literature on Kaseesa. However, authors have provided indications regarding Kaseesa in their own literatures.

**Rasa Tarangini (Sadananda Sharma):** Churna Kaseesa is pale yellow and white in colour. But Pushpa Kaseesa is clear green coloured.

**Dravya Guna Vigyana (Acharya Yadavji Trikamji):** It is bluish green coloured granular substances. When it is heated, water of crystallization evaporates and colour changes to white, which is also soluble in water.

**Pratyaksha Aushadhi Nirmana (Shri V. Dwivedi):** Ancient references on artificial preparation of Kaseesa are available. In all Rasa Granthas the description of preparation of Kaseesa is available. Kaseesa is prepared by chemically reacting iron and sulphur in the ratio of 2:3. It can also be prepared by reacting iron, sulphur and water in the ratio of 1:1:7. Kaseesa is available in four colours namely, yellow, black, red and white. They are known as Kaseesa, Dhatu Kaseesa, Pushpa Kaseesa and Panshu Kaseesa respectively.
**Pashchatya Dravya Guna Vigyana (Ramsushil Singh):** There are transparent and green crystals of Kaseesa or pale yellowish bluish green coloured powder, without any smell and astringent taste. If this is kept in open air, it absorbs moisture from air i.e. O2 and converts into ferric sulphate. Due to this it losses original colour and becomes yellowish. This type of Kaseesa is not used for medicinal purpose. This is soluble in water.

**Indian Materia Medica (Dr. Nadkarni):** It occurs as pale bluish green oblique rhombic prisms. Crude greenish blue crystals of sulphate of iron are available in Indian market. It tastes astringent like, is odourless, acidic in reaction, soluble in water and alcohol. It is valuable haematinic, tonic and is irritant to stomach.

**Sources of Kaseesa:** Kaseesa is obtained in natural form and is also prepared artificially, since ancient times. Nowadays, it is prepared by reacting sulphuric acid on big iron pieces.

**Natural Sources of Kaseesa:** Kaseesa is mostly obtained from iron ores and from places where Shilajit, Sphatika, Suvarna Makshika are available. Kaseesa is obtained in natural form where there is hot springs of sulphur. Thus sulphur reacts with iron in the earth and ferrous sulphate and other compound of sulphur are formed.

**Kaseesa is obtained in the following places in India:**

**Bihar and Orissa:** Near Rohitagarh, Kaseesa is found in the form of powder, which is whitish yellow in colour. It is mixed with sand and contains 39% Hirakasa, 36% Mandura
and 25% magnesium. It is mainly as paint. Along with this there are many factories which prepare artificial Kaseesa.

**Punjab:** Special clay which contains ‘Sphatika, Hirakasa and Aluminum is available in Punjab. From this clay, first Sphatika is separated and remaining contents are dried in sunlight. These dried contents have three types of colour, which contains Kaseesa in more or less percentage. They are known as –

- Kahi Sapheda: It contains Sphatika and Hira Kaseesa.
- Kahi Sabja: It is green coloured clay containing more percentage of Hira Kaseesa.
- Kahi Jarda: This is yellow coloured clay with large quantity of Pushpa Kaseesa.

**Uttar Pradesh:** The two rivers Ramganga and Garjaya in Almora and Mirjapur districts of Uttar Pradesh have, hot springs of sulphur. Hira Kaseesa is obtained near the banks of these riveres.

**Kashmir:** Colourful clay named as Kahi Siya and Kahi Sapheda are available in various regions of Kashmir. White coloured clay is used for colouring leather.

**Baluchistan:** Kaseesa is found on big stones containing sulphur and iron. This is called Malenite. The sample from this place contains 27-30% Hira Kaseesa and 4% aluminum. Near the mountain ‘Koi-E-Sultan’ we get special type of clay named as ‘Makaigiri’ containing Kaseesa and Sphatika. This type of clay is used for painting the houses.
**Origin of Kaseesa:** Mythological stories are described for origin of Rasa Dravyas. It seems that our ancient Acharyas were influenced by Pauranika thoughts. Though it is not possible to correlate these thoughts with modern science such stories on the origin of Parada, Vaikranta, and Sasyaka etc are available. But there is no description on the origin of Kaseesa. It may be because Kaseesa is not obtained in natural form abundantly. Kaseesa is prepared artificially. Most of the Rasashastra authors have adopted modern method. We can see reflection of this in the text Rasa Tarangini.

**Artificial Preparation of Kaseesa**

R.T. 21/239): Pure iron powder is taken in a glass container and dilute sulphuric acid is added drop by drop until the iron powder is dissolved completely. When sulphuric acid is added, iron powder becomes hot and froth is formed in the liquid. When froth stops, solution is filtered through filter paper. Fluid part is collected properly and same quantity of pure alcohol is added to it, so that precipitation takes place and Kaseesa gets settled at the bottom. Supernatant fluid is separated and Kaseesa which settles at the bottom is collected and dried in sunlight. After drying we get green coloured crystals of Kaseesa.

**Types of Kaseesa:** In Rasashastra, Rasa Dravyas are classified according to their colour, size, shape and origin. So we get types of Kaseesa according to colour and its origin.

**Samhita Kala:** In Charaka (Ch. Si. 6/64), Sushruta (Su.Su. 38/37) and Vagbhata have clearly mentioned that Kaseesa is of two types i.e. Kaseesa and Pushpa Kaseesa.

**Rasa Kaala:** Rasa Granthas like Rasa Tarangini (R. T. 21/228) mentioned two types of Kaseesa i.e. Kaseesa and Pushpa Kaseesa. In Rasa Ratna Samuchchaya (R.R.S. 3/52) Valu Kaseesa and Pushpa Kaseesa are of two types. Acharya Yadavji Trikamji in his
book Rasamrita has written two types like Pushpa Kaseesa and Valuka Kaseesa. Ayurved Prakash (A. P. 2) mentioned three type of Kaseesa i.e. Pushpa Kaseesaalong with Dhatu Kaseesa and Panshu Kaseesa. In Rasarnava and Brihad Rasa Raja Sundara three types of Kaseesa are named according to colour such as Shweta Kaseesa, Pitta Kaseesa and Krishna Kaseesa. The author of Rasa Jala Nidhi has mentioned four types of Kaseesa according to colour adding Harita Kaseesa in previous types. Hence some authors have classified Kaseesa into two categories whereas others have classified it into three or four categories.

**Grahya Agrahyat:** Many of the Rasa Dravyas have toxicity, so awareness of the drug is must. In Samhitaa clear description of GrahyaAgrahyata of Kaseesais unavailable. After screening all Rasa Granthas, it can be said that yellowish Kaseesa with a bluish tinge, one which is lusterous and is known as PushpaKaseesa, is used for the medicinal purpose in humans. In Charaka and Sushruta Samhita two types of Kaseesa are mentioned which were followed by Acharya Yadavaji Trikamji to render his opinion on artificially prepared Kaseesaus for therapeutic purpose. Valuka Kaseesa which is obtained in natural form i.e. dust form is not used for the medicinal purpose. Nowadays Kaseesa, which is sold in the market, is artificially prepared with iron and sulphuric acid. Kaseesa prepared with poor quality of iron does not yield satisfactory result.

**Kaseesa Shodhana:** Definition and importance of Shodhana are described earlier. However, various methods of Shodhana of Kaseesa are mentioned, amongst them Bhavana and Swedana methods are widely used. According to Rasamrita 3/158 that in Kaseesa Shodhana is done by three Bhavana of Bhringaraja Swarasa. For Swedana method, according to R. T. 21/250, Kaseesa Shodhana is done for three hours in Dolayantra containing Bhringaraja Swarasa.

**Rasapanchaka of Kaseesa:** The fact of Rasapanchaka is essential to understand how Kaseesa works in curing disease. Rasa of Kaseesa is mentioned as Amla and Kashaya by almost all authors. While Rasa Ratna Samuchchaya and Ayurveda Prakasha have mentioned as Kshara and Tikta Rasa respectively. All Rasagrantas have mentioned it’s
Guna as Ushna. But Rasa Jala Nidhi has mentioned Snigdha Guna while Shishira Guna is
told by Raj Nighantu. Most Rasacharya have not made explicit mention of its virya.
Only Rasa Ratna Samuchchayakara has taken Virya as Ushna and Rasa Jala Nidhikara as
Shita Virya. According to Rasa Ratna Samuchchaya and Rasamrit have mentioned Katu
Vipaka whereas Rasa Kamdhenu has mentioned it is Madhura.

**Guna Karma of Shuddha Kaseesa:** Rakta Sanjanan, Raja Pravartaka, Kesharanjana,
Ama Sanshoshana, Balya, Sankochaka, Shvitraghna, Netrya, Vishaghna, Vatamayahara,
Kacha Ranjana, Shleshmahara, Mutrakrichchhrahara, Kandughna, Pandughna,
Krimighna, Kashayaghna, Plihaghna, Kustaghna, Jwaraghna, Ashmarighna, Visarpa
Shothahara, Savarnakara, Danta Krimihara, Hikkahara, Vranaghna, Rasabandhana,
Tridoshahara, Apasmritighna, Pittaprashmanahara, Rasayana Gunakarakara.

**Kaseesa Marana:** Concept of Marana has been described earlier. In Rasa Grantha 17
methods of Kaseesa Marana are described. According to Rasamrit 3/158, Shodhita
Kaseesa is triturated with Nimbu Swarasa and incinerated with 10 Prastha of Vanyopala.
Puta should be given till Kaseesa Bhasma become tasteless i.e. Niramlatva. Rasa
Tarangini and Rasayana Sara have mentioned different fluid media like Kanji, Triphala,
Langali and Snuhipatra Swarasa.

**Guna Karma of Kaseesa Bhasma:** Indicated in Agnimandya, Arsha, Kashtartava, Guda
Bhramsha, Pandu, Shotha, Rajorodha, Yonivyapada

**Dose of Kaseesa Bhasma:**
- 65 mg – 250 mg Rasatarangini
- 125 mg – 250 mg Rasamrit, Dravya Guna Vigyana
- 125 mg – 375 mg Rasatantra Sara
Anupana: Kaseesa Bhasma should be given with the following Anupana.

Vataja Vyadhi Triphala Churna
Pittaja Vyadhi Sharkara
Kaphaja Vyadhi Madhu

Modern Review of Kaseesa (Ferrous Sulphate): Ferrous sulfate is the chemical compound with formula FeSO₄ 7 H₂O. It is used to treat iron deficiency and also for industrial applications. All iron sulfates dissolve in water to give rise to Fe and H₂O octahedral molecule.

Hydrates: Ironsulfate can be found in various states in nature.

- FeSO₄·5H₂O (mineral: Siderotil relatively rare)
- FeSO₄·6H₂O (mineral: Ferrohexahydrite relatively rare)
- FeSO₄·H₂O (mineral: Szomolokite relatively rare)
- FeSO₄·4H₂O (mineral: Rozenite white, relatively common, may be dehydratation product of melanterite)
- FeSO₄·7H₂O (mineral: Melanterite, blue-green, relatively common)
- The heptahydrate in solution transforms to tetrahydrate and heptahydrate at temperature 65 °C and at 55 °C then tetrahydrate and monohydrate are formed by both.

Production and reactions: Ferrous sulfate is prepared commercially by oxidation of pyrite:

\[ 2 \text{FeS}_2 + 7 \text{O}_2 + 2 \text{H}_2\text{O} \rightarrow 2 \text{FeSO}_4 + 2 \text{H}_2\text{SO}_4 \]

Uses: Nutritional supplement: Together with other iron compounds, ferrous sulfate is used to preservation of food and to treat anemia.
1.2.3 Rajapravartini Vati:

Rajapravartini Vati is a herbomineral preparation indicated in Rajo rodha and Kashtartava. It contains Shuddha Hingu, Shuddha Kaseesa, Shuddha Tankana and Kumari Swarasa in equal quantity (Aloe vera Linn). 3 ratti (375mg) weighing vati should be prepared. This formulation is also mentioned in Bharata Bhaishaja Ratnakara having similar ingredients but dose is mentioned as 2 ratti.

Method of preparations of Rajah Pravartini Vati

Reference: Bhaishajya Ratnavali Stri Rogadhikara & Ayurveda Sarasangraha

Rajah Pravartini Vati tablet ingredients:

Shatavari (Asparagus racemosus Wild)

Gajara Beeja (Daucus carota subsp. Sativus)

Karpasa (Gossypium herbaccum Linn)

Vamsha (Bambusa arundinacea Wild)

Bola (Commiphora myrrha)

Kumari (Aloe ver Tourn ex Linn)

Hingu (Ferula narthex Boiss)

Kaseesa (Ferrous Sulphate)

Tankana (Borax)

1 part fine powder of each ingredient and 2 parts of Bola triturated with Kumari Swarasa and tablets are made, dried and preserved

Ref – Ayurveda Sara Sangraha

Manufacturers: Dabur, Baidyanath, Dhoot Papeshwara Pvt Ltd.

Expiry date: Two years from the date of manufacture. However, it is advisable to consume the contents within one year of opening the container.
Shodana of Kaseesa,\textsuperscript{163} Hingu (Ferula narthex boiss) and Tankana\textsuperscript{164} as per classical reference. According to Modern Science and Ayurveda the Hing,\textsuperscript{165} Kaseesa\textsuperscript{166} Tankana and Kanyasara are usefil in Udavarta/kashtartava as one of the vataja yoni vyapat mentioned in all classics. In fact, as per Sushruta kashtarthaivas first yoni vyapat.\textsuperscript{167}

\textbf{Table - 3 Pharmacological Properties of ingredients of Rajapravartini Vati}

<table>
<thead>
<tr>
<th>Name of the ingredient</th>
<th>Latin Name / English Name</th>
<th>Pharmacological Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shatavari</td>
<td>Asparagus racemosus Wild</td>
<td>Shukrala, Garbha Poshaka, Stanya Janana</td>
</tr>
<tr>
<td>Gajara Beeja</td>
<td>Daucus carota subsp. sativus</td>
<td>Vit A, Calcium, Vit D, Vit –B12</td>
</tr>
<tr>
<td>Karpasa</td>
<td>Gossypium herbaccum Linn</td>
<td>Garbhashaya Sankochaka, Artava Janaka, Sthanya Janaka</td>
</tr>
<tr>
<td>Vamsha</td>
<td>Bambusa arundinacea Wild</td>
<td>Shothahara , Artava janana</td>
</tr>
<tr>
<td>Bola</td>
<td>Commiphora myrrha</td>
<td>Artava Janaka</td>
</tr>
<tr>
<td>Kumari</td>
<td>Aloe ver Tourn ex Linn</td>
<td>Rajorodha, Shukradourbalya</td>
</tr>
<tr>
<td>Hingu</td>
<td>Ferula narthex Boiss</td>
<td>Raja Krichra, Klaibya, Garbhashaya shodhaka</td>
</tr>
<tr>
<td>Kaseesa</td>
<td>Ferrous Sulphate</td>
<td>Raja Pravartaka, Sankochaka</td>
</tr>
<tr>
<td>Tankana</td>
<td>Borax</td>
<td>Arthava Janaka, Muda Garbha Pravrthaka</td>
</tr>
</tbody>
</table>

\textbf{Previous work done:}

1) Dr Mukhi (1995) - A Preparation of kumari Ghana kalpa and its clinical evaluation by comparing its efficacy with “Rajapravartini vati”- Jamnagar

2) Dr Kotbagi (1998)- To study efficacy of “Rajapravartini vati” in Rajo rodha – Pune

3) Dr Lambat Varsha (2003) - “Rajapravartini vati” nirmana – clinical evaluation in krichrartava – Nagpur
4) Dr Janapapiya (2003) – An analysis and comparative study of Jyotishmatyadi yoga and “Rajapravartini vati” in Arajaska yoni vyapat.- Kerala


6) A study done by Bhagyalakshmi B R et.al entitled Experimental Evaluation of Oestrogenic activity of Rajahpravartini vati - A Herbo-mineral formulation showed significant oestrogenic activity of Rajapravartini Vati at all dose levels. But lower doses were having significant oestrogenic activity in all parameters. Histo-pathologically the drug at low doses increased endometrial thickness in comparison with control group suggesting oestrogenic activity.

7) CCRAS Project Clinical evaluation of efficacy of Rajahpravartini Vati, Kanchanara guggulu and Varunadi Kashaya in the management of Polycystic Ovarian Syndrome (PCOS):
1.2.4 FALAA Gold

Falaa Gold is a precisely planned product. It helps to ensure smooth and comfortable pregnancy. It helps to reduce threatened and habitual abortion. It is rich source of essential micro minerals for better nourishment. Improves micro capillary circulation to uterine tissue resulting in enriched nutrition.

Each Falaa Gold Capsule Contains: 400mg

- Brayonia Lacinosa (Shivlingi Beej) 139 mg.
- Putranjiva Roxburgii (Putranjivika Beej) 100 mg.
- Garbhapala Ras 100 mg.
- Ficus Bengalensis (Plaksha Jata) 15 mg.
- Mor Pankh Bhasma 15 mg.
- Rajata Patra 5 mg.
- Cuminum Cyminum (Shweta Jeeraka) 10 mg.
- Mukta Pishti 5 mg.
- Pravala Pishti 10 mg.
- Gold Leaf 1 mg

**Dosage:** 2 Capsules along with cows milk, on empty stomach, for ten days. Care should be exercised to refrain from eating for two hours after consuming the medicine. During the treatment, advised to take milk, rice, fruits etc.
Table -4 Pharmacological Properties of Ingredients of Falaa Gold

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Latin Name / English Name</th>
<th>Pharmacological Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shivlingi Beej</td>
<td>Brayonia Lacinosa</td>
<td>Vandhyatwa</td>
</tr>
<tr>
<td>Putranjivika Beej</td>
<td>PutranjivaRoxburgii</td>
<td>Vandyatwa</td>
</tr>
<tr>
<td>Vata(Komal Bargad )Jata</td>
<td>Ficus bengalensis Linn</td>
<td>Garbhashaya shotha hara, Shukra stambaka</td>
</tr>
<tr>
<td>Shweta Jeeraka</td>
<td>Cuminum cyminum Linn</td>
<td>Garbhashaya shotha hara, Stanya Janana, Vrushya</td>
</tr>
<tr>
<td>Rajata</td>
<td>argentum</td>
<td>Rasayana, Vrushya, Virya, Bala , Ayushya etc</td>
</tr>
<tr>
<td>Garbhpal Ras</td>
<td>HerbominerlPreparation</td>
<td>Protects Garbha from Garbha srava &amp; Pata</td>
</tr>
<tr>
<td>Mayura Piccha Bhasma</td>
<td>Peacock Feather Ash</td>
<td>Brimhana,Amla Pitta, Shoola nshak</td>
</tr>
<tr>
<td>Mukta Pishti</td>
<td>Pearl</td>
<td>Vrushya, Virya, Bala , Ayushya etc</td>
</tr>
<tr>
<td>Pravala Pishti</td>
<td>Coral</td>
<td>Shukrala , Vrushya, Balya</td>
</tr>
<tr>
<td>Swarna</td>
<td>aurum</td>
<td>Increases Kama Shakti, Pumsavana, Garbhadharana,Vrushya etc</td>
</tr>
</tbody>
</table>
1.2.5 Madhu (Honey)

**Historical Review:** In Ayurveda Madhuis described under Madhu Varga\textsuperscript{168} and Ikshu Varga\textsuperscript{169, 170} which is one of the products of animal origin. Madhuis formed from multiple compounds and is useful in various diseases. It is known as Yogavahi.\textsuperscript{171, 172} Hence, it is mentioned as anupana of most of ayurvedic formulations. Proper quantity of madhu is effective for maintainence of health whereas it’s extensive use products Amaajirna\textsuperscript{173}.

**Synonyms:**\textsuperscript{174} Bhrigavant, Kshaudra, Kusumasava, Makshika, Madhvika, Makarandarasa, Pitrya, Pavitra, Saraga, Pushpasava, Pushparasahyva, Varativant etc.

**Classification:**\textsuperscript{175} According to Charaka- 4 types.

Makshik- Shrestha, Tailavarnam

Bharamar- Guru, Shweta varnam

Kshudra- Kapilvarnam

Paitikka- Ghritavarnam

**Other classification and their therapeutic use:**\textsuperscript{176}  

1. Nava Madhu- Brimhana, Pushtrakara, Kinchita Kaphahara, Saraka
2. Puran Madhu- Grahi, Ruksha, Lekhana, Sthaulyakara
3. Pakwa Madhu- Tridishahara
4. Aama Madhu- Amla Rasa and tridoshakrit.
Contemporary view Honey: Honey a naturally occurring sweet fluid produced by honeybees by enzymatic transformation of floral nectar ingested by them and deposited in cells of hives or combs. It is produced by honey bees of genus Apis.

Types of Honey:
1. Apiary honey- transparent and adulterant free
2. Forest honey- Prepared by rock bee. Pollen, wax, brood etc. present as adulterant.

Description: A thick syrupy translucent yellow to yellowish brown fluid, sweet with a pleasant odour and flavour.

Storage: Honey should be stored preferably at 20 °C - 25°C away from heat and should not be refrigerated.

Honey analysis

- Fructose: 36-38%
- Glucose: 30-32%
- Maltose: 6-7%
- Sucrose: 0.5-1%
- Water: 15-16%
- Higher Sugars: 1-2%
- Ash: 0.3-0.4%
- Other/undetermined: 3-4%
- Honey has a density of about 1.35 kilograms per litre (35% denser than water).
Researches on Honey:

1. Madhu is having properties like phagocytosis, detoxification and proteolysis, all of which assist in cleaning of wounds.\textsuperscript{178}

2. It has excellent property to heal the wound by virtue of its Shodhana (Purification), Ropana(Healing) and Sandhana(Union) actions.\textsuperscript{179}

3. Coniferous and thyme honey showed highest antibacterial activity with minimum dilution of 17\% and 19\% (w/v) followed by citrus and polyfloral honeys with 20\% and 23\% respectively.\textsuperscript{180}

4. Honey consumption delays the postprandial ghrelin response (p=0.037), enhances the total PYY (p=0.007) response and meal induced thermogenesis responsible in part for the potential obesity protective effects and blunt glucose response (p=0.039) compared with consumption of sucrose containing meal.\textsuperscript{181}

5. Honey is more effective in reducing frequency of cough.\textsuperscript{182}

6. The presence of Nerve Growth Factor (NGF) was detected in snake, bee and scorpion venoms.\textsuperscript{183}

7. Sackel has observed that Bacillus, Microccus and Saharomytes species could be readily isolated from honey combs and adult bees.\textsuperscript{184}
8. Bacillus spp are most prevalent followed by Mould, Actinomycetes, Gram negative rods (probably of Enterobacteriaceae) and yeast have also been isolated while streptomyces spp were recovered from one larva.\textsuperscript{185}

9. The intestines of bees has been found to contain 1% yeast, 27% gram positive bacteria including Bacillus, Bacteridium, Streptococcus and Clostidiumspp 70% gram negative bacteria or gram variable bacteria including Achromobacter, Citrobacter, Proteus and Pseudomonas.\textsuperscript{186}

10. Most of the bacteria and microbes do not grow in honey. As it is having antibacterial activity.\textsuperscript{187}

11. Aristotle, when discussing different honeys, referred to pale honey as good as a salve for sore eyes and wounds.\textsuperscript{188}

12. Mast cell degranulating peptide (MCDP): This peptide is a basic 22 amino acid residue component of honey bee toxin with unusual immunological and pharmacological activities. Mast cell degranulating peptide is a potent anti inflammatory agent, but at low concentration it is strong mediator of mast cell degranulation and histamine release.\textsuperscript{189}
1.2.6 Draft OECD Guideline for the Testing of Chemicals\textsuperscript{190}

The Uterotrophic Bioassay in Rodents: A short-term screening test for oestrogenic properties

**DESCRIPTION OF THE METHOD**

**Animal Ethical Committee Approval** - Should be taken

**Procurement of animals**- Animals should be procured from a licensed breeder/Institution.

**Selection of animal species**- Wistar strains of rats should be used.

**Quarantine**- Received animals should be kept in quarantine for 1-3 days.

**Number and condition of animals**- Treated and control group should include 6 immature female rats.

**Age of immature animals**: For the Uterotrophic Bioassay with immature animals the day of birth should be specified. Dosing should begin early enough to ensure that the physiological rise of endogenous oestrogens connected with puberty has not yet taken place. As per test guideline, dosing in rats can be started immediately after birth on postnatal day 17-18 (with the day of birth being postnatal day zero). Dosing in rats preferably should be completed on postnatal day 20-21 but prior to postnatal day 24-25, since, after this period, the hypothalamic-pituitary-ovarian axis becomes efficient and endogenous oestrogen levels may begin to rise with an increase in baseline uterine weight suggest an increase in the group standard deviations \((2)(3)(10)(11)(12)\).

**Housing and feeding conditions**

**Light**: The daily lighting sequence should be 14 hour light /10 hour dark.
Ventilation: Adequate ventilation should be maintained for proper oxygen supply.

Temperature: The temperature in the experimental room should be 20°C-23°C. The relative humidity should be a minimum of 30% to 70%.

Noise: Noise in animal rooms should be avoided since rats may experience reproductive problems when exposed to excessive noise.

Identification: Identification should be done by a cage label which includes title of the protocol, the name and phone number of the investigator, species, and strain, sex, age and source etc.

Cage Space: 3-4 rats should be stored in each cage.

Bedding Material: Bedding material should be clean, waterless, free from dust, spongy, harmless and preferably soft & Bedding must be changed when it becomes wet.

Social Behavior: Animals should be group housed and should be assessed for compatibility and separated if there is significant aggression.

Sanitation: Cages should be washed by using detergent and water. After washing bleach water is used for sanitization. The procedure is done once a week or if required many times.

Food: Laboratory diet should be provided *ad libitum.*

Water: Tap water from potable water from faucet available at all times.

Preparation of animals: Experimental animals not having of any visible physical abnormalities and disease are randomly divided into the control and trial groups. The animals should be identified by marking. The acclimatization period prior to the start of
the study should be about 5 days for young adult animals and one day for the immature animals delivered with dams or fosters dams.

**Body weight:** In immature rat body weight is related to uterine weight. Therefore, at the commencement of the study the weight variation of should not exceed ± 20% of the mean weight. The weight of the animal should be standardized by the supplier by feeding same amount of food to all animals born to different mothers. Care should be taken that mean body weight of each group should not different from any other group statistically.

**Dosage:** Test group and a control group should be handled in similar manner. If a vehicle is used in administering the trial drug, the highest volume of used with the test groups should be given to control group. The control group should receive the Human dose mentioned in the classical texts of Ayurveda are converted into animal dose based on their body wt and administered to the rats.

**Administration of doses:** The test compound should be administered by oral gavage. Single daily dose to the animals using suitable intubation cannula.

**Observations:**

**General and clinical observations:** General clinical observations should be made minimum once a day. If required many times. Observations should be carried out preferably at the same time(s) every day and considering the period of expected peak effects after dosing. All animals should be observed for death, morbidity and general
clinical signs such as changes in behaviour, skin covering, hair, eye, mucous membranes, lacrimation, piloerection, pupil size, unusual respiratory pattern and excreations.

**Body weight and food consumption:** All animals should be weighed daily to the nearest 0.1 g, before starting the treatment. As an optional measurement, the amount of food eaten by animals during the treatment period may be measured per cage. The food consumption results should be expressed in grams per rat per day.

**Euthanasia:** As per CPCSEA guidelines.

**Dissection:** 24 hours after the last dose, the rats should be humanely sacrificed. As per the bioassay both the wet and blotted uterus weights should be measure. The wet weight includes the contents of uterus and the luminal fluid. Before dissection the vagina should be examined for opening. The dissection is started by opening the abdominal wall starting at the pubic symphysis. Uterus and ovaries are removed from the abdominal wall. The urinary bladder, ureters are separated uterus and vagina. The uterus and vagina are detached. The uterus should be detached carefully cutting the uterine mesentery. Ovaries are present, the ovaries are removed at the oviduct avoiding loss of luminal fluid from the uterine horn. Excess fat and connective tissue should be trimmed away as shown in Figure 2. Seperated uterus of each animal should be transferred to a pre marked container and weighed (e.g. a petri-dish or plastic plate). The uterus with luminal fluid should be weighed to the nearest 0.1 mg (wet uterine weight).

**Waste management:** As per Waste management rules all animal waste as well as other wastes should be done through proper channel.
DATA AND REPORTING

Study data should include:

The number of animals: The number and identity of animals died during the study or sacrificed date and time should be noted. The animals showing signs of toxicity, including time of onset, duration, and severity of any toxic effects etc should be noted.

A final report should include:

Testing facility: Responsible personnel and their study responsibilities

Test Substance: Characterization of test substances and Physico chemical properties

Vehicle: Name of vehicle. Justification for vehicle (in case other than water)

Test animals: Species, strain, Supplier name, Age on supply with birth date, Details of animal accommodation procedure, Details of number of animals per cage, Details of identification

Assay Conditions:
- Details of randomization process (i.e., method used)
- Details of test substance administration
- Diet
- Watersource
- Type of bedding
- Lighting interval
- Room temperature and humidity
- Room cleaning
- Detailed description of Sacrifice
- Uterus weighing procedure
- Statistical procedure
Results

For individual animals:

- Every day body weights of all animals
- Before starting the treatment age of the animal in days.
- Duration and time of drug administration
- Recording every day status of animal
- The cause of death during study period.
- Date and time of humane killing with time interval to last dosing
- Wet uterine weight
- Dissection

For each group of animals:

- Mean body weights
- Mean wet uterine weights (to the nearest 0.1 mg)
- Food consumption calculated in grams per animal.
- The results of statistical analysis comparing the total body weight and the body weight gain of treated groups relative to the same measures in the vehicle control groups.

Table - 5 Summary of the important guidance facts of the Test Guideline

<table>
<thead>
<tr>
<th></th>
<th>Rat</th>
<th>Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strain</td>
<td>Commonly used laboratory rodent strain</td>
<td></td>
</tr>
<tr>
<td>Number of animals</td>
<td>A minimum of 6 animals per dose group</td>
<td></td>
</tr>
<tr>
<td>Number of groups</td>
<td>4 test groups and a control groups see paragraphs</td>
<td></td>
</tr>
<tr>
<td>Housing and feeding conditions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T° in animal room</td>
<td>22°C ± 3°C</td>
<td></td>
</tr>
<tr>
<td>Relative humidity</td>
<td>50-60% and not below 30% or above 70%</td>
<td></td>
</tr>
</tbody>
</table>
### Daily lighting sequence

12 hours light, 12 hours dark

### Diet and drinking water

Ad libitum

### Housing

Individually or in groups of up to three animals (social group housing is recommended for immature animals)

### Diet and bedding

Low level of phytooestrogens recommended in diet and bedding

## Protocol

<table>
<thead>
<tr>
<th>Method</th>
<th>Immature non-ovariectomised method (the preferred one).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of dosing for immature animals</td>
<td>PND 18 at the earliest. Dosing should be completed prior to PND 25</td>
</tr>
<tr>
<td>Body weight</td>
<td>In the immature model, body weight variation should be minimal and not exceed ± 20% of the mean weight.</td>
</tr>
</tbody>
</table>

## Dosing

<table>
<thead>
<tr>
<th>Route of administration</th>
<th>Oral gavage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency of administration</td>
<td>Single daily dose</td>
</tr>
<tr>
<td>Volume amount for</td>
<td>≤ 5ml/kg body weight (or up to 10 ml/kg body weight in case of</td>
</tr>
</tbody>
</table>
**Review of Literature**

<table>
<thead>
<tr>
<th>gavage and injection</th>
<th>aqueous solutions)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Duration of administration</strong></td>
<td>3 consecutive days for immature model</td>
</tr>
<tr>
<td><strong>Time of necropsy</strong></td>
<td>Approximately 24 hours after the last dose</td>
</tr>
</tbody>
</table>

**Results**

| Positive response | Statistically significant increase of the mean uterus weight (wet and or blotted) |

**GUIDANCE FOR THE INTERPRETATION AND ACCEPTANCE OF THE RESULTS:** In general, a test for oestrogenicity should be considered positive if there is a statistically significant increase in uterine weight (p < 0.05) as compared to the solvent control group. A positive result is further supported by the demonstration of a biologically possible relationship between the dose and the magnitude of the response, bearing in mind that overlapping oestrogenic and antioestrogenic activities of the test chemical may affect the shape of the dose-response curve. Care should be taken in order to allow a meaningful interpretation of the data. Reduction of body weight, clinical signs, and other findings should be thoroughly assessed in this respect.

An important consideration for the acceptance of the data from the Uterotrophic Bioassay is the uterine weights of the vehicle control group. High control values may compromise the responsiveness of the bioassay and the ability to detect very weak oestrogen agonists. Literature reviews and the data generated during the validation of the Uterotrophic Bioassay suggest that instances of high control means do occur spontaneously,
particularly in immature animals (2)(3)(6)(9). As the uterine weight of immature rats depends on many variables like strain or body weight, no definitive upper limit for the uterine weight can be given. As a guide, if blotted uterine weights in immature control rats are comprised between 40 and 45 mg, results should be considered as suspicious and uterine weights above 45 mg may lead to rerun the test. However, this needs to be considered on a case by case basis (3)(6)(8).

Blotted vehicle control uterine weights less than 0.09% of body weight for immature female rats appear to yield acceptable results [see Table 31 (2)]. If the control uterine weights are greater than these numbers, various factors should be scrutinized including the age of the animals, dietary phytooestrogens, and so on, and a negative assay result (no indication for oestrogenic activity) should be used with caution.

Historical data for vehicle control groups should be maintained in the laboratory. Historical data for responses to positive reference oestrogens, such as 17α-ethinyl estradiol, should also be maintained in the laboratory. Laboratories may also test the response to known weak oestrogen agonists. All these data can be compared to available data (2)(3)(4)(5)(6)(7)(8) to ensure that the laboratory’s methods yield sufficient sensitivity.

The blotted uterine weights showed less variability in the course of the OECD validation study than the wet uterine weights (6)(7). If divergent results are obtained by the blotted versus the wet uterine weights, the blotted weights should be given preference for the
The uterotrophic response is not entirely of oestrogenic origin, however, a positive result of the Uterotrophic Bioassay should generally be interpreted as evidence for oestrogenic potential in vivo, and should normally initiate actions for further clarification (see paragraph 9 and the “OECD Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals”)

**Figure 1: Schematic Diagram showing the Surgical Removal of the ovaries**

The procedure begins by opening dorso-lateral abdominal wall at the mid point between the costal inferior border and the iliac crest, and a few millimetres lateral to the lateral margin of the lumbar muscle. Within the abdominal cavity, the ovaries should be located. On an aseptic field, the ovaries are then physically removed from the abdominal cavity, a ligature placed between the ovary and uterus to control bleeding, and the ovary detached by incision above the ligature at the junction of the oviduct and each uterine horn. After confirming that no significant bleeding persists, the abdominal wall should be closed by suture, and the skin closed, e.g., by autoclips or suture. The animals should be allowed to recover and the uterus weight to regress for a minimum of 14 days before use.
Figure 2: The Removal and Preparation of the Uterine Tissues for Weight Measurement.

The procedure begins by opening the abdominal wall at the pubic symphysis. Then, each ovary, if present and uterine horn is detached from the dorsal abdominal wall. Urinary bladder and ureters are removed from the ventral and lateral side of uterus and vagina. Fibrous adhesion between the rectum and the vagina are detached until the junction of vaginal orifice and perineal skin can be identified. The uterus and vagina are detached from the body by incising the vaginal wall just above the junction between perineal skin as shown in the figure. The uterus should be detached from the body wall by gently cutting the uterine mesentery at the point of its attachment along the full length of the dorsolateral aspect of each uterine horn. After removal from the body, the excess fat and connective tissue is trimmed away. If ovaries are present, the ovaries are removed at the oviduct avoiding loss of luminal fluid from the uterine horn. If the animal has been ovarectomised, the stubs should be examined for the presence of any ovarian tissue. The vagina is removed from the uterus just below the cervix so that the cervix remains with the uterine body as shown in the figure. The uterus can then be weighed.
1.2.7 Follicle Stimulating Hormone (FSH)

**Follicle Stimulating Hormone (FSH)** is present in humans and also in animals. Anterior pituitary gland helps to produce and secrete gonadotrops FSH.\(^{191}\) It regulates the development, growth, puberty and fertility activities of the body. FSH secretion produces ovulation.

**Structure:** Glycoprotein is present in FSH. Each unit is a protein attached with a sugar. FSH structure looks like HCG, LH and TSH. Two polypeptides of the protein are labeled as Alpha and Beta. Alpha subunit having ninety two amino acids. Beta subunit varies. Beta subunit contains one hundred and eleven amino acids, which performs special biological action and it interacts with the FSH-receptor. Sugar part of the FSH contains fructose, galactose, sialic acid, mannose, galactosamine and glucosamine. Half-life of FSH is 3–4 hours.

**Genes:** The alpha subunit gene is present on chromosome 6p21.1-23. The Beta subunit gene is present on chromosome 11p13. It is expressed in pituitary cells of gonadotrops and is controlled by GnRH.

**Activity in Females**

- Stimulates for the maturation of germ cells.
- Starts follicular growth by affecting granulosa cells.

Blood content ranges of follicle-stimulating hormone levels during the menstrual cycle.\(^{192}\)

The **Inter-woman variability** are appropriate where the average cycle lengths, time of ovulation are not known.
Measurement: Follicle stimulating hormone is measured in follicular phase of the menstrual cycle i.e day three to five, counted from last menstruation. At this time, the oestradiol (E2) levels and progesterone are lowest.

FSH Levels: FSH levels are present but low during childhood and high after menopause.

High FSH Levels: High FSH levels during the reproductive age is not normal. High FSH levels may include premature menop, turner syndrome, premature ovarian aging etc

Low FSH Levels: Decreased FSH secretion can result cessation of reproductive cycles. The condition which may cause low FSH levels are- Polycystic Ovarian Syndrome, Hypothalamic suppression, Hypopituitarism, Hyperprolactinemia etc

Potential Role in Vascularization of Solid Tumors: Elevated FSH receptors are detected in the wide range of solid tumors. Use of FSH and FSH-receptor antagonists acts as an anti tumor angiogenesis therapy.\(^{196}\)
1.2.8 Luteinizing Hormone

Luteinizing Hormone (LH) is called as Lutropin\textsuperscript{197} and Lutrophin.\textsuperscript{198} It is secreated by anteriorpitutary glands gonadotroph cells. Sudden increase in the level of LH stimulates ovulation\textsuperscript{199} and growth of the corpus luteum. LH in male is called Interstitial Cell-Stimulating Hormon.\textsuperscript{200}

**Structure:** Glycoprotein is present in LH. The structure looks like HCG, LH and TSH. Two polypeptides of the protein are labeled as Alpha and Beta. Alpha subunit having ninety six amino acids. Beta subunit varies. Beta subunit contains one hundered and twenty amino acids, which performs special biological action and it interacts with the LH-receptor. Sugar part of the FSH contains fructose, galactose, sialic acid, mannose, galactosamine and glucosamine. Half-life of LH is 20 minutes.

**Genes:** The alpha subunit gene is present on chromosome 6q12.21. The Beta subunit gene is present on chromosome 19q13.32. It is expressed in pituitary cells of gonadotrops and is controlled by GnRH.

**Activity in Females**

- Stimulates for the maturation of germ cells.
- Starts follicular growth by affecting granulosa cells.

Blood content ranges of follicle-stimulating hormone levels during the menstrualcycle.\textsuperscript{192}

The **Inter-woman variability** are appropriate where the average cycle lengths, time of ovulation are not known.
Activity In females: FSH starts follicular growth by affecting granulosa cells. With the rise in estrogens, stimulation of LH receptors causes maturation of follicle. It lasts for 24 to 48 hours.

Normal Levels

During the reproductive years levels are between 1-20 IU/L.

Predicting Ovulation

LH can be identified by using urinary ovulation kits (OPK), done daily near expected ovulation time to perform artificial inseminisation etc with the intentions of conception.

Luteinizing hormone testing may be combined with estrodiol tests.

High LH Levels: Seen in premature menopause, polycystic ovary syndrome, congenital adrenal hyperplasia etc

Low LH Levels: Seen in Hypothalamic suppression, Hypopitutarism, Eating disorder
1.2.9 Namburi Phased Spot Test:204

The 'Namburi Phased Spot Test' is a new technique developed by Dr. Hanumantha Rao after a lot of trial and error. It is an identifying technique for various bhasma and Sindura Kalpanas. In this method, Whatman paper No. 1 is invariably impregnated in a suitable reagent and dried. Unlike the conventional method of spottest, in this new technique, the spot is not rejected immediately after treating and noting the chemical reaction. As long as the reaction continues, the spot is studied at three different time intervals, viz. 1st phase or Immediate Reaction (within five minutes after treatment) 2nd Phase or Delayed Reaction (between five and twenty minutes) and 3rd Phase or Late Reaction (beyond 20 minutes to some hours).

Some Special features of this technique:

- Study of the spot at three different time intervals.
- Pattern of colour display of the spot.

Materials and Methods:

- Whatman filter paper No. 1 square pieces 12 cm x 12 cm.
- Distilled water
- Capillary or a pipette
- Test tubes
- Glass rods and glass sheet
- Volumetric flask of 100 ml capacity.
Reagents:

- 5 N HNO3
- 10% Potassium iodide solution.
- 10 g of Potassium iodide dissolved in 100 ml of distilled water.
- The cut pieces of Whatman filter paper are to be dipped in the Potassium iodide solution and left for drying on a glass sheet after complete soaking.
- Due care to be taken to avoid tearing, wrinkling of the paper or formation of bubbles underneath it during drying.
- The papers usually dry within 2 to 2 1/2 hours.

Procedure for Spotting:

- The impregnated papers need to be arranged on the glass sheet and two drops of the clear supernatant solution are dropped at the centre of the paper from a suitable height.
  
- Development of the spot is then carefully observed in natural day light and observations to be noted.

A Gross Division of Spot Areas: Central Zone: The central area of the spot, measuring about 1 cm in diameter. This may be marked by a boundary or may have dispersed edge. Middle Zone: The area just external to the central zone may again to be extended 1cm centrifugally. Peripheral Zone: The exterior most zone with or without sharp boundaries.
1.2.10 X-Ray Diffraction Method: It is categorized as a special and sophisticated technique, conducting the analysis in a non-destructive fashion. A variety of X-Ray techniques and methods are in use. The main three categories in which all the methods are classified are:

i. X-Ray Absorption Methods

ii. X-Ray Fluorescence Methods

iii. X-Ray Diffraction Methods

The details of X-Ray Diffraction method:

Principle: When a beam of X-radiation is incident upon a substance, the electrons constituting the atoms of the substances become as small oscillators. These oscillate at the same frequency as that of incident X-radiation. These scattered waves come from electrons which are arranged in a regular manner in a crystal lattice and then travel in certain directions. Every crystalline substance scatters the X-rays in its own unique diffraction pattern produces a finger print of its atomic and molecular structure. The following methods are used in the X-ray Diffraction Technique.

i. Laue Photographic Method

ii. Bragg X-ray Spectrometer Method

iii. Rotating Crystal Method

iv. Powder Method
In the Bragg X-ray Spectrometer method: When X-rays fall on a sample, they get diffracted as per the Bragg's equation: \( n \lambda = 2d \sin \theta \) Where, \( \lambda \) = Wavelength of X-rays, \( d \) = Spacing between the layers of atoms, \( \theta \) = Angle of incident X-rays.

**Sample Preparation:**

The powdered sample is placed in a sample holder and analysis carried out in a static position with the detector moving through \( 2 \theta \) 3 to 70.

**Characterization:**

The X-ray diffraction of the sample is matched against the standard reference spectra library of software for phase identification. The method gives certain emission peaks which are characteristic of elements contained in the target. The wavelengths of the peaks can be related to the atomic number of the elements producing them, so they provide a means of identifying elements present in the target sample. Further more, under controlled conditions, the intensity of the peaks can be used to determine the amounts of the different elements which are present. This is the basis of "electron probe micro analysis", in which a small target area of the sample in pinpointed for examination. This has important applications in metallurgical research and in determining the metallic elements in biological materials (if present).
1.2.11 Scanning Electron Microscope (SEM):

The SEM is a type of microscope where electrons are used. There are many advantages in using the SEM instead of a light microscope. It is designed for direct studying of the surfaces of solid objects. The Electron beam created by electron is used for scanning, an image is formed. SEM is able to produce three dimensional image of the sample.

Heating of metallic string, beam of electrons produced on the top of the microscope. The electron beam follows a vertical path from column of the microscope. It makes its way from electromagnetic lenses which focuses, direct the beam down towards the sample. Once sample gets heated, the other electrons are ejected from the sample.

Detectors collect the scattered electrons convert them in to a signal and send screen similar to the one in an ordinary television, producing an image.
1.2.12 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy, also known as FTIR Analysis. This method identifies organic, polymeric, inorganic materials. Infrared beam is used in FTIR analysis.

Valuable analysis with FTIR Spectroscopy

- Assessing purity

- Identifying:
  - Base polymer composition
  - Additives
  - Organic contaminants
  - General type of material being analyzed when there are unknowns

Analyzing samples in a variety of forms

- Solids placed on a crystal

- Liquids placed between two sodium chloride plates

- Thin film placed in a cassette

FTIR spectroscopy helps in identifying material characterization, qualitative and quantitative analysis of element.
The Test Process

FTIR analysis enough sample is required to obtain an absorption spectrum. First collection of a background spectra and then subtraction from the test spectra. Next, the sample is analyzed, which produces the absorbance spectra showing the unique chemical bonds and the molecular structure. This profile is in the form of an absorption spectrum, peaks represent the higher concentration. Functional groups are indicated by absorbance peaks on the spectrum e.g. alkanes, ketones, acid chlorides. Different types of bonds form different functional group based on different wavelengths. The analysis is done in absorbance.

The spectrum is then compared with reference library program with cataloged spectra to identify components. Identification of organic, polymeric and inorganic including sample composition, contamination and unknown materials

- Analysis performed in absorbance or transmittance
1.2.13 Reproductive system in Albino Rats

The female reproductive system is in such a way that it produces, transports and provides favorable environment for fertilization and development of embryos at proper time. The reproductive system also provides nourishment to the growing fetus and produces female sex hormones. The structures of the female reproductive system of the rat are the vagina, ovaries, uterus, and mammary glands.

Reproductive organs

Vagina: Vagina is the small muscular canal that runs from the female rat’s uterus to the outside the body. It is just below the urethra. The vaginal walls are protected and moist due to mucous membranes. The vagina helps in birth and helps for the receiving of sperm during sexual activity. Soon after birth female rats vagina will be closed. When rats grow 33-42 days old it ruptures on its own.

Ovaries: At the distal end of the uterine horns the ovaries are located near the kidneys. The ovaries connected to horn of the uterus through oviducts. The ovaries produce hormones and the ova.

Uterus: The uterus provides facility to eggs and nourishes the growing embryos development up to birth. Rats have a uterus consisting of the right and left horns. This structure enables the rat to have multiple off spring .The horns of the uterus form the vagina.
Mammary Glands: Alveoli are present in Mammary glands lined with milk producing epithelial cells. Through the holes of nipples the milk drains from the lobules to the lactiferous ducts. Due to the contraction of mammary tissue milk is pushed from the alveoli to the nipples. The mammary glands grows through several phases. At birth the epithelium is inside the fat and formed with a ductal tree where several ducts and lateral branches originating from each primary duct.

Cervix: Uterine horn has cervix. Each cervix is located at the utero-vaginal junction. Each cervix has strong thick walls. Though the opening of the cervix is too small but extends during birth.

Clitoris: In Rats clitoris connects to the urethra rather than to the vagina and situated above the urethra.

Preputial Glands (clitorial glands): Exocrine glands present in the female rat are called preputial glands. Situated near the clitoris which produce pheromones. A pheromone is a chemical produced by a living human being to attract the male.

Physiology of human Menstruation: Menstruation is monthly uterine bleeding for 4-5 days coming regularly every 28 days from puberty till menopause. This is normal uterine function.

The Estrous Cycle in rats: Estrus was used first used by Heape. Derived from Latin adopted Greek word oistros. It means gadfly, sting, or frenzy to describe the Freemanin 1994 explained duration of sexual interest in certain stages such as:

Anestrous: Non breeding period, when reproductive organs are inactive

Proestrus: when animal nearing the sexually active
Metestrus: in the absence of conception estrus changes in the reproductive tract disappear

Diestrus: Preparation of reproductive tract takes place for receiving the ovum

The puberty starts in the female rat after development of luteinizing hormone (LH) and its release after the fourth week after birth (approximately 30 days of age). Then ovarian maturation (Andrews and Ojeda 1981) occurs. Before LH release the reproductive tract is dormant. LH release is perceptible eight to nine days before the first proestrus. The first proestrus, estrus, and diestrus periods then starts (Advis et al. 1979; Ojeda et al. 1976).

Ovulation occurs in the youngster laboratory rat every four to five days once throughout the year (Ojeda and Urbanski 1994). The normal length of the estrous cycle is identified based on the researches carried out so far. To study the length of estrous cycle Long and Evans (1922) used approximately two thousand young rats. In bread crossed between several white females. They measured cycle lengths from three to thirty-eight days. They excluded the rats whose cycle was more than 8 days, the average was 4.8 days. Based on vaginal smears, the duration and the stages of the estrous cycle for rats with a four- or five-day cycle are proestrus- 13-14 hours, estrus- 25-26 hours, Metestrus- 6-7 hours and diestrus- 56-57 hours. (Astwood, 1939, Hartman 1944, Long and Evans 1922; Mandl 1951).
1.3 JUSTIFICATION FOR THE STUDY

In Ayurveda herbs, minerals, metals, marine originates etc are used to treat diseases. A single drug mentioned in Ayurveda has many applications. Depending on different disease conditions same drug may act differently, when given along with proper Anupana and pathya. Many popular Ayurvedic drugs such as Swarna bhasma, Kaseesa bhasma etc. have varied properties attributed to them. Today's pharmacologist says his product is evidence-based while that of the traditional vaidya is empirical.

Utilizing and integrating novel analytical methods, technologies that enable to study effects of Ayurvedic treatments.

Many of the assumptions being made about Ayurvedic medicine "in the west" are not simply based on reliable and accessible information.

Generating scientific analytical, experimental and clinical data from ancient Ayurvedic principles and conveying research output to the public in an easily understandable and accessible way.

The present study has attempted to evaluate the effects or the mode of action of four Ayurvedic formulations i.e. Swarna Bhasma, Swarna containing compound formulation, Kaseesa Bhasma and Kaseesa containing compound formulations on Reproductive System of the animal model.
2. MATERIAL AND METHODS

Materials and Methods include following 3 steps

1. Selection of drugs for the study

2. Analytical study

3. Experimental study

**Selection of drugs for the study:**

Primary Selection of Swarna Bhasma and Kaseesa Bhasma is done on classical bhasma parikshas and Namboori Phased Spot test. The methodology followed for each bhasma is explained below.

### 2.1.1 Methodology of Selection of SwarnaBhasma

3 Samples of Swarna Bhasma were purchased from local Ayurvedic Medical shop (Manufactured by GMP Certified Ayurvedic drug manufacturer) subjected to the following classical bhasma pareeksha (Analysis)

**Bhasma Pareeksha**

**Practical Number 1**

**Name of the Test** – Varitara *(Ref: RRS 8/26)*

**Aim**- To test Varitara of selected bhasma

**Instruments and Equipments**- Weighing machine, 100ml capacity 3 glasses, 3 spatulas, 3 plates
Ingredients and Quantity

Swarna Bhasma - Sample 1, Sample 2 and Sample 3 sufficient quantity.

Potable water – for each sample 100 ml X 3 samples

Procedure - Sufficient quantity of 3 Samples of Swarna Bhasma were sprinkled over a glass containing 100 ml of water carefully.

Observation – Sample 1, 2 and 3 passed Varitara Pariksha

Practical Number 2

Name of the Test – Rekhapurna (Ref: RRS 8/27)

Aim - To test Rekhapurna of selected bhasma

Instruments and Equipments- Weighing machine, 3 spatulas and 3 plates

Ingredients and Quantity

Swarna Bhasma - Sample 1, Sample 2 and Sample 3 sufficient quantity

Procedure - Sufficient quantity of Swarna Bhasma of each sample were rubbed in between thumb and index finger.

Observation – Sample 1, 2 and 3 Passed Rekhapurna Bhasma Pariksha.

Practical Number 3

Name of the Test – Unam /Uttama test for Bhasma (Ref: RRS 8/29)

Aim - To test unam /uttama test for selected bhasma.

Instruments and Equipments- Weighing machine, 100ml capacity 3 glasses, 3 spatulas, 3 plates
Materials and Methods

Effect of Swarna Bhasma and Kaseesa Bhasma on Reproductive Function in Female Rats

Ingredients and Quantity

Swarna Bhasma - Sample 1, Sample 2 and Sample 3 sufficient quantity.

Rice grain – 2-3 Rice Grain for each sample x 3

Potable water – 100 ml x3

Procedure – Sufficient quantity of Swarna Bhasma was sprinkled over a glass containing 100 ml of water; 2-3 grains of rice were placed over the floating bhasma.

Observation – All the 3 samples Passed Unam Bhasma Pariksha.

Practical Number 4

Name of the Test – Apunarbhava (not regaining its metallic state) (Ref: RRS 8/28)

Aim- To test Apunarbhava of selected bhasma

Instruments and Equipments- Weighing machine, muffle furnace, 3 spatulas, 3 plates, 3 Musha.

Ingredients and Quantity

Swarna Bhasma - Sample 1, Sample 2 and Sample 3 Sufficient quantity of each

Guda– Each sample 1 Part x3

Gunja- Each sample 1 Part x3

Tankana-Each sample 1 Part x3

Madhu - Each sample 1 Part x3

Gritha- Each sample 1 Part x3

Procedure –1 part of Swarna Bhasma was mixed with 1 part of Mitrapanchaka (Guda, Gunja, Tankana, Madhu and Gritha) and subjected to heat.

Observation – All the 3 samples Passed Apunarbhava Bhasma Pareeksha.
Practical Number 5

Name of the Test – Niruttha (RRS 8/30)

Aim- To test niruttha of selected bhasma

Instruments and Equipments- Weighing machine, muffle furnace, 3 spatulas, 3 plates, 3 crucibles.

Ingredients and Quantity

Swarna Bhasma - Sufficient Quantity
Silver - Sufficient Quantity.

Procedure-Sufficient Quantity of Swarna Bhasma was placed with Sufficient Quantity of silver piece and subjected to heat in a crucible.

Observation – All the 3 samples passed Nirutta Bhasma Pareeksha

Practical Number 6

Name of the Test – Sukshma and Slakshna (Anubhuta)

Aim- To test sukshmatwa and slakshnata of selected Bhasma

Instruments and Equipments- Weighing machine, 3 spatulas, 3 plates, 3 crucibles

Ingredients and Quantity

Swarna Bhasma - Sample 1, Sample 2 and Sample 3 of sufficient quantity

Procedure-1mg Swarna Bhasma was rubbed in-between thumb and index finger to feel the sukshmatwa just like talc powder.

Observation - By touch, talc like softness was felt in all the 3 samples.
Practical Number 7

Name of the Test – Swadarahita (Nihswadu) (Ref: Ayurvediya Rasashastra by Siddhinadan Mishra)

Aim- To test Swadarahita (Tasteless) of selected Bhasma

Instruments and Equipments- Weighing machine, 3 spatulas, 3 plates

Ingredients and Quantity

Swarna Bhasma - Sample 1, Sample 2 and Sample 3 of sufficient quantity.

Procedure – Sufficient quantity of Swarna Bhasma was placed on the tip of the tongue with the help of thumb and index finger.

Observation – Samples 1 and 3 passed Nihswadu Bhasma Pariksha. But Sample 2 was having slight metallic taste.

Practical Number 8

Name of the Test – Nirdhumatwa (Ref: Ayurvediya Rasashastra Paribhasha prakarana by Siddhinadan Mishra)

Aim- To test nirdhumatwa of selected bhasma

Instruments and Equipments- Weighing machine, charcoal, 3 spatulas, 3 plates, 3 Mushi.

Ingredients and Quantity

Swarna Bhasma - Sample 1, Sample 2 and Sample 3 of sufficient quantity each

Procedure- Sufficient quantity of Swarna Bhasma was sprinkled on red hot charcoal.

Observation – Sample 1 passed Nirdhuma Paiksha. Sample 2 and 3 failed.
Practical Number 9

Name of the Test – Nischandra (Ref: RRS2/93)

Aim- To test Nischandra of selected bhasma

Instruments and Equipments- Weighing machine

Ingredients and Quantity

Swarna Bhasma - Sample 1, Sample 2 and Sample 3 sufficient quantity of each

Procedure- Sufficient quantity of Swarna Bhasma was rubbed inbetween thumb and index figure and observed in sunlight for Chandrika (Shine).

Observation – All the 3 samples passed Nishchandra Bhasma Pariksha.

All the 3 Samples of Swarna Bhasma were subjected to Namburi Phased Spot Test.
Swarna Bhasma Namburi Phased Spot Test (NPST) 207

Pre operative Procedure

10% Pot Iodide paper preparation

Materials

- 10% Pot. Iodide solution  -90 ml
- Whatman’s filter paper -09

Procedure

- 10% Pot.Iodide solution was taken in a 9 clean watch glasses.
- In each watch glass 10 ml 10% Pot.Iodide solution was taken.
- 9 Whatman’s filter papers where dipped in watch glass containing 10 ml 10% Pot.Iodide solution for 5 seconds.
- After confirming uniform spreading of Pot.Iodide solution papers were immediately removed and kept for drying on glass slab for 14 hrs

Operative Procedure

Method: Phased Spot Test

Materials:

- Aqua Regia
- 10% Pot. Iodide paper
- Test tubes
- Samples of Swarna Bhasma
Materials and Methods

Procedure

110 mg of each sample of Swarna Bhasma was taken in a test tube and 2 ml of Aqua Regia and heated till the reaction starts. After 24 hours, solution was diluted with 2 ml of distilled water. The solution was put on the reaction paper after 48 hours.

1. Quantity of Bhasma: Each 110 mg
2. Reagents: Aqua Regia
3. Heating: Each sample was heated 30 min after treating with its reagents till reaction starts. The samples were cooled and allowed 48 hours for reaction.
4. After 24 hours the solution was diluted with 2 ml of distilled water.
5. Solution ready for use: After 48 hours, the solution was ready to study on 10% Pot. Iodide paper.

Post Operative Procedure

Observation and inferences on various chemical reacting papers Swarna Bhasma. A deep brick red central solid spot with deep brick red margin.

The Sample 1 showed standard classical Bhasma Pareeksha and NPST Results was selected

Details of SwarnaBhasma Sample Selected for the Study

Swarna Bhasma

Manufactured by SDL

Batch no – P120500101

Mfg Date- May 12

Exp Date- 5 years
Materials and Methods

Methodology Used by the Shri Dhoot Papeshwara Pvt Ltd to prepare Swarna Bhasma

Ref – Bharata Bhaishajya Ratnakara 5/8357

Shuddha Swarna 1 Part + Shuddha Parada 2 Parts and bhavana with nimbuSwarasa then formation of lingakara, Shuddha Gandhaka 3 Parts spread around lingakara in Sharava and finally Kukkuta Puta were given for 14 times peak temp observed was 650°C.
2.1.2 Methodology of Selection of Kaseesa Bhasma

3 Samples of Kaseesa Bhasma were purchased from local Ayurvedic Medical shop (Manufactured by GMP Certified Ayurvedic drug manufacturer) and subjected to following classical bhasma pareeksha (Analysis)

**Kaseesa Bhasma Pareeksha**

**Practical Number 1**

**Name of the Test** – Varitara (Ref: RRS 8/26)

**Aim**- To test Varitara of selected bhasma

**Instruments and Equipments**- Weighing machine, 100ml capacity 3 glasses, 3spatulas, 3 plates

**Ingredients and Quantity**

Kaseesa Bhasma - Sample 1, Sample 2 and Sample 3 sufficient quantity.

Potable water – for each sample 100 ml X 3samples

**Procedure** - Sufficient quantity of 3 Samples of Kaseesa Bhasma were sprinkled over a glass containing 100 ml of water carefully.

**Observation** – Sample 1and 2 passed Varitara Pariksha. But Sample 3 did not pass this Bhasma Pariksha.

**Practical Number 2**

**Name of the Test** – Rekhapurna (Ref RRS 8/27)

**Aim**- To test rekhapurna of selected bhasma

**Instruments and Equipments**- Weighing machine, 3 spatulas and 3 plates
Materials and Methods

**Ingredients and Quantity**

Kaseesa Bhasma - Sample 1, Sample 2 and Sample 3 sufficient quantity

**Procedure** - Sufficient quantity of Kaseesa Bhasma of each sample were rubbed in between thumb and index finger.

**Observation** – Sample 1, 2 and 3 Passed Rekhapurna Bhasma Pariksha.

**Practical Number 3**

**Name of the Test** – Unam /Uttama test for Bhasma (Ref: RRS 8/29)

**Aim** - To test unam /uttama test for selected bhasma.

**Instruments and Equipments**- Weighing machine, 100ml capacity 3 glasses, 3 spatulas, 3 plates

**Ingredients and Quantity**

Kaseesa Bhasma - Sample 1, Sample 2 and Sample 3 sufficient quantity.

Rice grain – 2-3 Rice Grain for each sample x 3

Potable water – 100 ml x3

**Procedure**– Sufficient quantity of Kaseesa Bhasma was sprinkled over a glass containing 100 ml of water; 2-3 grains of rice were placed over the floating bhasma.

**Observation** – All the 3 samples Passed Unam Bhasma Pariksha.

**Practical Number 4**

**Name of the Test** – Apunarbhava (not regaining its metallic state)

**Aim**- To test Apunarbhava of selected bhasma
Materials and Methods

**Effect of Swarna Bhasma and Kaseesa Bhasma on Reproductive Function in Female Rats**

**Instruments and Equipments**
- Weighing machine, muffle furnace, 3 spatulas, 3 plates, 3 Musha.

**Ingredients and Quantity**
Kaseesa Bhasma - Sample 1, Sample 2 and Sample 3 Sufficient quantity of each

Guda– Each sample 1 Part x3

Gunja- Each sample 1 Part x3

Tankana-Each sample 1 Part x3

Madhu-Each Sample1Partx3

Gritha- Each sample 1 Part x3

**Procedure** – 1 part of Kaseesa Bhasma was mixed with 1 part of Mitrapanchaka (Guda, Gunja, Tankana, Madhu and Gritha) and subjected to heat.

**Observation** – All the 3 samples Passed Apunarbhava Bhasma Pareeksha.

**Practical Number 5**

**Name of the Test** – Niruttha (Ref: RRS 8/30)

**Aim** - To test Niruttha of selected bhasma

**Instruments and Equipments**- Weighing machine, muffle furnace, 3 spatulas, 3 plates, 3 crucibles.

**Ingredients and Quantity**

Kaseesa Bhasma - Sufficient Quantity

Silver - Sufficient Quantity.

**Procedure**- Sufficient Quantity of Kaseesa Bhasma was mixed with Sufficient Quantity of silver piece and subjected to heat in a crucible.

**Observation** – All the 3 samples passed Nirutta Bhasma Pareeksha
Practical Number 6

Name of the Test – Sukshma and Slakshna (Ref: Anubhuta)

Aim- To test sukshmatwa and slakshnata of selected Bhasma

Instruments and Equipments- Weighing machine, 3 spatulas, 3 plates, 3 crucibles

Ingredients and Quantity

Kaseesa Bhasma - Sample 1, Sample 2 and Sample 3 of sufficient quantity

Procedure- Kaseesa Bhasma was rubbed in-between thumb and index finger to feel the sukshmatwa just like talc powder.

Observation - By touch, talc like softness was felt in Sample 1 and 2. But, sample 3 failed this test.

Practical Number 7

Name of the Test – Swadarahita (Nihswadhu) (Ref: Paribhasha Prakarana Ayurvediya Rasashastra by Dr Sisshinadan Mishra)

Aim- To test Swadarahita (Tasteless) of selected Bhasma

Instruments and Equipments- Weighing machine, 3 spatulas, 3 plates

Ingredients and Quantity

Kaseesa Bhasma - Sample 1, Sample 2 and Sample 3 of sufficient quantity.

Procedure – Sufficient quantity of Kaseesa Bhasma was placed on the tip of the tongue with the help of thumb and index finger.

Observation – Samples 1 and 3 passed Swada Rahita Bhasma Pariksha. But Sample 2 was having Slight sour taste.
Practical Number 8

Name of the Test – Nirdhumatwa (Ref Paribhasha Prakarana Ayurvediya Rasashastra by Dr Sisshinadan Mishra)

Aim- To test nirdhumatwa of selected bhasma

Instruments and Equipments- Weighing machine, charcoal, 3 spatulas, 3 plates, 3 Musha.

Ingredients and Quantity

Kaseesa Bhasma - Sample 1, Sample 2 and Sample 3 of sufficient quantity each

Procedure- Sufficient quantity of Kaseesa Bhasma was sprinkled on red hot charcoal.

Observation – Sample 1 and 3 passed NirdhumaPaiksha. But Sample 2 failed.

All three samples of Kaseesa Bhasma were subjected to Namburi Phased Spot Test (NPST).
Kaseesa Bhasma Namburi Phased Spot Test (NPST)\textsuperscript{207}

Pre operative Procedure

10% Pot Iodide paper preparation

Materials

- 10% Pot. Iodide solution - 90 ml
- Whatman’s filter paper - 09

Procedure

- 10% Pot.Iodide solution was taken in a 9 clean watch glasses.
- In each watch glass 10 ml 10% Pot.Iodide solution was taken.
- 9 Whatman’s filter papers where dipped in watch glass containing 10 ml 10% Pot.Iodide solution for 5 seconds.
- After confirming uniform spreading of Pot.Iodide solution papers were immediately removed and kept for drying on glass slab for 14 hrs

Operative Procedure

Method: Phased Spot Test

Materials:

- 5\text{n} HNO\textsubscript{3}
- 10% Pot. Iodide paper
- Test tubes
- Samples of Kaseesa Bhasma
Procedure

0.25 gm of each sample was taken in a test tube and 0.5 ml of 5N HNO₃ was added to one set and 5% HCL v/v to another set of samples.

1. Quantity of Bhasma: Each 0.25 gms

2. Reagents: 5NHNO₃ and 5% HCl v/v

3. Heating: Each sample was heated in the test tube till the bottom appeared red before treating with its reagents. The samples were cooled and treated with the reagents and heated again for a while. Then they were shaken now and then till two hours, before they were treated with the chemical reacting paper.

4. Time allowed to react: 10% Pot. Iodide paper the bhasma were allowed to react with their reagents for 40 hours and 72 hours

5. Solution ready for use: After 72 hours, the solution was ready to study on 10% Pot. Iodide paper.

Post Operative Procedure

Observations and inferences on various chemical reacting papers Kaseesa Bhasma with 5NHNO₃. A deep blue solid spot was formed without blue periphery. A blue margin encircles the entire spot very closely.

Sample 1 showed standard results of bhasma pareeksha and standard NPST was selected.
Details of Kaseesa Bhasma Sample Selected for the Study.

Kaseesa Bhasma

Manufactured by KLE Ayurved Pharmacy

Batch no – 100165

Mfg Date- 12/10Exp Date- 5 years

Method adopted by KLE Ayurveda Pharmacy to prepare Kaseesa Bhasma

Ref – Siddha Yoga Sangraha

Grahya Kaseesa - 1 part bhavana with Amalaki Swarasa and Bhringaraja Swarasa, preparation of chakrikas and subjected to Ardha Gaja Puta two times. The peak temp observed was 1008°C
2.1.3 FALAA GOLD (Swarna Containing Compound Formulation)

Swarna containing compound formulation FALAA GOLD was purchased from the local market, manufactured by GMP Certified Pharmacy.

Following are the Details of Swarna Bhasma Containing Compound Formulation Selected for the Study.

FALAA GOLD

Manufactured by BAN

Batch no - 113

Mfg Date - May 12

Exp Date - 4 years from manufacturing

Each FALAA Gold Capsule Contains: 400mg

Brayonia Lacinosa (Shivlingi Beej) 139 mg.

Putranjiva Roxburgii (Putranjivika Beej) 100 mg.

Garbhpal Ras 100 mg.

Ficus Bengalensis (KomalBargadJata) 15 mg.

Mor Pankh Bhasma 15 mg.

ChandiWaraq 5 mg.

Cuminum Cyminum (Safed Jeera) 10 mg.

Moti Pisti 5 mg.

Praval Pisti 10 mg.

Gold Leaf 1 mg
2.1.4 Rajapravartini Vati (Kaseesa Containing Compound Formulation)

Kaseesa containing compound formulation Rajapravartini Vati was purchased from the local market, manufactured by GMP certified ayurvedic pharmacy.

Following are the Details of Kaseesa Containing Compound Formulation Selected for the Study.

Rajapravartini Vati

Manufactured by SDL
Batch no – DU311105
Mfg Date- November 2011
Exp Date- October 2016
Ref – Ayurveda Sara Sangraha

Rajah Pravartini Vati tablet ingredients:

Shatavari (Asparagus racemosus Wild) - 1 part
Gajara Beeja (Daucuscarota subsp. Sativus) - 1 part
Karpasa (Gossypium herbaccum Linn) - 1 part
Vamsha (Bambusaarundinacea Wild) - 1 part
Bola (Commiphoramyrhrha) - 2 part
Hingu (Ferula narthex Boiss) - 1 part
Kaseesa (Ferrous Sulphate) - 1 part
Tankana (Borax) - 1 part
Kumari (Aloe veraTourn ex Linn) – qs for Bhavana

1 part fine powder of each ingredient and 2 parts of Bola triturated with Kumari Swarasa, paste is prepared and tablets are made, dried and preserved
2.1.5 Analytical Study

All the selected drugs for the study i.e Swarna Bhasma, Kaseesa Bhasma, FALAA Gold and RajahapravartiniVati were sent to IIT Mumbai for ICPAES for elemental analysis and FTIR to know different groups present, XRD analysis for form, SEM analysis to know the particles size to Sophisticated Test and Instrumentation Centre (STIC) Cochin University, Kochi, Kerala. The methods adopted by the centres to perform above mentioned analysis are explained below.

**Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES)**

Make: Thermo Electron Corporation

Model: IRIS INTREPID II XSP DUO

The atomic spectrum emitted by a sample is used to determine its elemental composition. The wavelength at which emission occurs identifies the element, while the intensity of the emitted radiation quantifies its concentration.

All the measurement are carried out in axial view. Spectral range: 170 to 800nm.

**Analysis Protocol**

The analytical practice would consist of first preparing a series of working standard solutions from ICP single/ multi elements 1000ppm standard solution. The working standard would cover the range of concentrations anticipated for the elements to be measured.
Materials and Methods

Argon gas used for create plasma. Ignite plasma and keep the instrument at least 15 minutes to allow the instrument to become thermally stable. Create a method in the instrument software suitable for the elements to be quantified. Calibration is done with blank and working standard solutions. Calibration linearity was verified for each element and should be greater than 0.998. After the calibration cleaning the sample introduction system with blank and checking the calibration with known standard solution.

The unknown sample solutions can be run and concentration equivalents for signals obtained calculated by the software. Three repeats were followed for each sample run. Appropriate dilution was done to bring the metal concentration to calibrated range.

**ICP AES Standard**

Working standard used for calibration instrument – 0.1ppm, 0.5ppm, 1ppm, 5ppm and 10ppm

Description of CRM: ICP Multi – element standard solution IV- 1000ppm NIST Traceable

Manufacturer: Merck.

**FTIR (Fourier Transform Infrared Spectroscopy)**

A small quantity of the sample was added to KBr in the ratio 1:100 approximately. The matrix was grind for 3-4 minutes using mortar and pestle. The fine powder is transferred into 13 mm diameter die and made into a pellet using a hydraulic press by applying a
pressure of 7 tonnes. The fine pellet was subjected to FTIR analysis using universal pellet holder. (a single drop of oil is poured on the KBr pellet in case of liquid samples)

Infrared spectral data were collected on Thermo Avtar 370 FTIR spectrometer.

Spectra are collected over a range of 4000–400 cm\(^{-1}\) at 4 cm\(^{-1}\) resolution with an interferogram of 32 scans.

Make /Model: Thermo Avtar 370 DTGS

XRD Analysis

The sample was smeared over low back ground sample holder (amorphous silica holder) and fixed on the sample stage in goniometer. The instrument was set with B-B geometry. The current and voltage is set to 40 mV and 35 mA and data was collected.

Instrument: Make Bruker Model D8 Advance

SEM analysis

The sample was smeared on a small piece of adhesive carbon tape which is fixed on a brass stub. The sample, then subjected to gold coating using sputtering unit (model: JFC1600) for 10 sec at 10mA of current. The gold coated sample placed in chamber of SEM (Jeol, JSM 6390LA) and secondary electron/Back Scattered electron images were recorded.

Make Model: Jeol, JSM 6390LA
2.1.6 Experimental Study

Experimental study was carried out to assess the effect of Swarna bhasma, Kaseesa bhasma, Swarna compound formulation and Kaseesa compound formulation by using Uterotrophic Bio Assay.

Description of the Method

Institutional Animal Ethical Committee Approval: Approval was taken from Institutional Animal Ethical Committee with approval letter No BMK/IAEC/Res-13/2012.

Guidelines: OECD Uterotrophic Bio Assay Draft Guideline for the testing of chemicals

Procurement of Animals: Animals were procured from JNMC animal house (licensed breeder/ Institution) Licence No- 627/02/a/CPCSEA Dated 19/06/2002

Age of Immature Animals: Wistar strains albino rats early weaning on postnatal day 18 (with the day of birth being postnatal day 0) were used.

Quarantine: Received animals were kept in quarantine for 1 day as per test guideline.

Number and Condition of Animals: Each treated and control group had 6 immature female rats. A total of 30 rats were studied in 5 groups.

Housing and Feeding Conditions:

Light: The daily lighting sequence was 14 hour light /10 hour dark

Ventilation: Adequate ventilation was maintained for proper oxygen supply
**Temperature:** The temperature in the experimental animal room was 22°C (with an approximate range ± 3°C). The relative humidity was minimum of 30% and preferably it did not exceed a maximum 70%, other than during room cleaning.

**Noise:** Noise in animal rooms was avoided since rats may experience reproductive problems when exposed to excessive noise.

**Identification:** Identification was done by a cage card which includes title of protocol, the name and phone number of the investigator, species, strain, sex, age and source.

**Cage Space:** 3-6 rats were stored in each cage.

**Bedding Material:** Bedding material was clean, dry, dust-free, absorbent, non-toxic and preferably soft and Bedding was changed every day.

**Social Behavior:** Animals were group housed and were assessed for compatibility and separated if there is significant aggression.

**Sanitation:** Cages were hand washed with detergent, rinsed in water, then dipped in a bleach containing water which acts as sanitizing agent and allowed to dry weekly or more often if required.

**Food:** Laboratory diet was provided *ad libitum.*

**Water:** Potable water from faucet available at all times was provided.

**Preparation of Animals:** Experimental animals not having any physical abnormalities or disease were randomly assigned to the control and treatment groups. Cages were arranged in such a way that possible effects due to cage placement were minimized. The animals were identified uniquely by marking with picric acid.
Body Weight: At the commencement of the study the weight variation did not exceed ±20 % of the mean weight. This means that the litter size was standardized by the breeder, to assure that offspring of different mother animals were fed approximately the same.

Grouping: Animals were randomly divided into 5 groups. Each group containing 6 animals by randomized weight distribution, so that mean body weight of each group is not statistically different from any other group.

Group 1- Received Honey (Control Group)

Group 2- Received Swarna Bhasma (SB Group) + Honey.

Group 3- Received Swarna Containing Compound formulation i.e FALAA GOLD (SCFGroup) + Honey.

Group 4- Received Kaseesa Bhasma + Honey (KB Group).

Group 5- Received Kaseesa Containing compound formulation i.e Rajapravartini Vati (KCF Group) + Honey.

Dosage: Except for treatment with the test substance, animals in the control group were handled in a similarly to the test group. Human dose mentioned in the classical texts of Ayurveda were converted into animal dose by referring table of Paget’s and Burnes 1969. Dosing in rats started on postnatal day 18 and completed on postnatal day 21.

Route of Administration: Oral

Time and Duration of Dosing: Once a day for 4 days
Observations

General and Clinical Observations: Clinical observations were made at least once a day and more frequently when required. Observations were carried out preferably at the same time every day and considering the period of expected peak effects after dosing. All animals were observed for mortality, morbidity and general clinical signs such as changes in behaviour, skin layer, hair, eye, mucous membranes, watering from eyes, piloerection, pupil size and respiratory pattern.

Body Weight and Food Consumption: All animals weighed every day to the nearest 0.1 g, before starting the treatment i.e., when the animals were allocated into groups. The amount of food consumed during the treatment period was also measured per cage by weighing the feeders. The food consumption results were expressed in grams per rat per day.

Euthanasia: As per CPCSEA guidelines.

The Removal and Preparation of the Uterine Tissues for Weight Measurement: The procedure began by opening the abdominal wall at the pubic symphysis. Then, each ovary, uterine horn was detached from the dorsal abdominal wall. The uterus and vagina were detached. The vagina was removed from the uterus just below the cervix. The uterus was then weighed.

Collection of Blood Sample: Twenty-four hours after the last treatment, under anesthesia through retro orbital puncture the blood was collected and transferred to pre labeled sterile bottles.
Vaginal Cornification: The animals were observed once daily for vaginal opening. Vagina was examined for opening status before starting the dissection.

Dissection and Measurement of Uterus Weight: Twenty-four hours after the last treatment the rats were humanely sacrificed. Uterus and ovaries were collected. Uterus was weighed before transferring into the pre-labeled and prefilled with formalin solution containers.

Parameters Measured

Body Weight: The body weight of rats were recorded once daily.

Uterus weight: Uterus was weighed with digital weighing machine.

Estimation of FSH and LH Hormone levels: Serum samples were stored at -20° C until assayed. Concentrations of Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) were estimated. The hormonal analysis was carried out by using RIAKEY FSH and LH IRMA Tube in RIA Laboratory Belagavi.

Organ weight: Ovaries and uterus were excised and weighed

Histology: Ovaries and uterus were serially sectioned (10 mm) and ovulation rate were confirmed histologically by counting the corpora lutea (CL) in the ovaries.

Waste Management: As per waste management rules all animal waste as well as other wastes disposed as per guidelines.
2.1.6 (A) Follicle Stimulating Hormonal Analysis

Follicle Stimulating Hormonal Analysis was carried out in RIA Laboratory, Belagavi by using RIAKEY FSH IRMA KIT.

Principle of the Assay:

The RIAKEY FSH IRMA is a one step noncompetitive immunoradiometric (IRMA) method (‘Sandwich’). The present method employs two highly specific monoclonal anti-FSH antibodies which recognize two different epitopes of the molecule. The one antibody is coated on solid phase (coated tube), the other, for the FSH labeled with iodine-125, is used as a tracer. Antibody coated polystyrene tubes serve as solid phase. The tracer antibody and the coated antibody react concurrently with the FSH antigen present in the standards, control serum and samples. Directly proportional to the antigen concentration in and the end of the incubation, the unbound material is removed. The radioactivity in the tubes is measured in a gamma counter.

Table - 6
Reagents Provided in RIAKEY FSH IRMA KIT:

<table>
<thead>
<tr>
<th>Kit Contents</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Coated Tubes</td>
<td>100 Test Tubes (50EA/rack x 2)</td>
</tr>
<tr>
<td>Main Anti – FSH monoclonal antibody</td>
<td></td>
</tr>
<tr>
<td>2. $^{125}$I Tracer</td>
<td>12ml x 1 vial, Ready to use</td>
</tr>
<tr>
<td>Main Anti – FSH monoclonal antibody labeled with $^{125}$I</td>
<td></td>
</tr>
<tr>
<td>Radio activity : 592 KBq</td>
<td></td>
</tr>
<tr>
<td>Diluent solution : BSA in PBS</td>
<td></td>
</tr>
<tr>
<td>Preservative : Sodium Azide</td>
<td></td>
</tr>
<tr>
<td>3. Standards</td>
<td>0.5ml x 7 vials Ready to use</td>
</tr>
<tr>
<td>Main : FSH of each conservation (Range : 0,2,5,25,50,100,200 mIU/ml)</td>
<td></td>
</tr>
<tr>
<td>Diluent solutions : BSA in BPS</td>
<td></td>
</tr>
<tr>
<td>Preservative : Sodium Azide</td>
<td></td>
</tr>
<tr>
<td>4. Control Serum</td>
<td>0.5ml x 1 vials Ready to use</td>
</tr>
<tr>
<td>Main FSH of 8-12 mIU/ml</td>
<td></td>
</tr>
<tr>
<td>Diluent Solutions : BSA in BPS</td>
<td></td>
</tr>
<tr>
<td>Preservative : Sodium Azide</td>
<td></td>
</tr>
</tbody>
</table>
Materials and Methods

- In Vitro Diagnostics Kit, 100 tests or 500 tests
- Store the kit at 2-8°C
- Unused tube should be store at 2-8°C in the appropriate bag with silica gel and tightly sealed
- The validity of each reagent is indicated on each vial.

Other Materials Required:

- Plastic test tubes (12 x75mm) and test tube rack.
- Adjustable automatic micropipettes.
- Cylinder and distilled water.
- Dispenser vortex mixer.
- Horizontal shaker capable of 300rpm and gamma counter.
- Aspiration pump or automated tube washing device.

Precautions:

Handling Precautions

- Do not use mixed reagents from different lots.
- Do not use reagent beyond the expiration dates.
- Use distilled water stored in clean container.
- Use an individual disposable tip for each sample and reagent to prevent the possible cross contamination among the sample.
- Store the unused coated tube at 2-8°C accurately sealed in appropriate bags with silica gel.
Materials and Methods

- If large quantity assay would be performed at one time, there might be substantial time variation between first and last tube tested, which may cause the error in the data. Therefore do not exceed 60 tubes at one time to minimize time variation. Also do not exceed 10 minutes for entire pipetting.

Sample Collection and Preparation:

- The assay can be performed directly on human serum or plasma. But highly lipemic or hemolyzed samples should be discarded.
- If the assay is performed within 1-2 days, the sample may be kept at 2-8\(^{\circ}\)C. For longer period, it is advisable to freeze sample at -20\(^{\circ}\)C.
- Fibrin filaments may exist in the plasma sample and it could interfere the whole assay. Therefore it is necessary to centrifuge the samples right before testing.

Procedure:

- To prevent any cross contamination among samples, every individual disposable tip should be used for each sample.
- The regents must be warmed up at room temperature of 18-25\(^{\circ}\)C at least for 30 minutes prior to use.
- Shake sample carefully prior to use. If the sample is frozen it must be homogenized by homogenizer, prior to use.

Method:

- Prepare and label the coated tubes for standards in duplicate per concentration, control serum in duplicate or more samples in single.
**Materials and Methods**

- Pipette 25ul of standards, control serum and sample (s) in to each different coated tube.
- Add carefully 100µl of $^{125}$I tracer in to all coated tube.
- Mix thoroughly and cover the tube with laboratory film or aluminum foil.
- Shake in 300rpm at room temperature (18-25°C) for 45min.
- Aspirate all liquid from the tubes.
- Wash the tubes 2 times with 2 ml of distilled water per tube.
- By the radioactivity in the tubes is measured in a gamma counter. To ensure the validity of $^{125}$I tracer, count the total radioactivity in the plain test tube filled with 100ul of $^{125}$ I tracer.

**Summary of Procedure:**

- Pipette 25ul of standards, control serum and sample (s) in to each different coated tube.
- Add carefully 100µl of $^{125}$I tracer in to all coated tube.
- Mix thoroughly and cover the tube with laboratory film or aluminum foil.
- Shake in 300rpm at room temperature (18-25°C) for 45min.
- Aspirate all liquid from the tubes.
- Wash the tubes 2 times with 2 ml of distilled water per tube.
- By the radioactivity in the tubes is measured in a gamma counter. To ensure the validity of $^{125}$I tracer, count the total radioactivity in the plain test tube filled with 100ul of $^{125}$ I tracer.

2ml x 2 times Aspiration and Washing (Distilled Water)

The whole assay procedures from 1) to 7) can be automated by BRIORIA

**Note:** For reassaying sample with concentrations greater than 200 mlU/ml, dilute the sample an additional 1:5 or 1:10 using the saline. Multiply by the dilution factor to obtain concentration of the sample.
Interpretation of the Results

Count the radioactivity of the standard, control serum samples and calculate their binding percent. B/Bmax(%)=Solidphasecpm/Standard 200(mIU/ml) cpmx100('%).

Use Precautions

- Wear disposable gloves while handling the kit reagents and wash handstherefully afterwards.
- Do not smoke, eat or drinkin areas where specimens or kit reagents are handled and do not pipette by mouth.
- Handle samples, reagents, and laboratory equipments used for assay with extreme care, as they may potentially contain infectious agents.
- Avoid microbial contamination when the reagent vial be eventually opened or the contents be handeled.
2.1.6 (B) Luteinizing Hormonal Analysis

Luteinizing Hormonal Analysis was carried out in RIA Laboratory, Belagavi by using RIAKEY LH IRMA KIT.

**Principle of the Assay:** The RIAKEY LH IRMA is a one step non-competitive immuno radiometric (IRMA) method (Sandwich). The method employs two highly specific monoclonal anti-LH antibodies which recognize two different epitopes of the molecule. One anti body is coated on solid phase (coated tube), the other specific for the LH and labeled with iodine -125, is used as a tracer. Antibody coated polystyrene tubes serve as solid space. The tracer antibody and the covered antibody react at the same time with the LH antigen present in the standards, control; serum and samples. Unbounded material is removed by washing. The amount of bound tracer isbe directly proportional to the LH antigen concentration and the remaining radioactivity bound to the tubes is measured in a gamma scintillation counter.

| Table -7 |
|-----------|-----------------|
| **Reagents Provided in RIAKEY LH IRMA KIT:** | |
| **Kit Contents** | **Quantity** |
| 1. Coated tubes | 100 Test tubes  
Main Anti – LH monoclonal antibody  
Diluent solution :PBS  
Protein Stabilizer :BSA | (50EA/rack x 2) |
| 2. Tracer | 08 ml x 1vial, Ready- to –use  
Main anti – LH monoclonal antibody labeled with 125I  
Radio activity :  799 KBq  
Diluent solution : BSA in PBS  
Preservative : 0.1% sodium Azide | |
| 3. Standards | 1 ml x 7 vials  
Main : LH of each conservation  
Diluent solutions : BSA in PBS  
Preservative : 0.1% sodium Azide | Range : 0,2,5,20,50,100,200mlU/ml  
Ready to use |
| 4. Control serum | 1ml x 1 vials  
Main LH of 8-12 mlU/ml  
Diluent solutions : BSA in PBS  
Preservative : 0.1% sodium Azide | Ready to use |
Other Material Required:

- Plastic test tubes (12 x75mm) and test tube rack
- Adjustable automatic micropipettes with disposable tips
- Graduated cylinder and distilled water
- Dispenser vortex mixer
- Horizontal shaker capable of 300rpm and gamma counter
- Aspiration pump or automated tube washing device

Precaution:

- Do not use mixed reagents from different lots.
- Do not use reagent beyond the expiration dates.
- Use distilled water stored in clean container.
- Use an individual disposal tip for each sample and reagent to prevent the possible cross contamination among the sample.
- Store the unused coated tube at 2-8°C accurately sealed in appropriate bags with silica gel.
- If large quantity assay would be performed at one time, there might be substantial time variation between first and last tube tested, which may cause an error in the data. Therefore do not exceed 60 tubes at one time to minimize time variation. Also do not exceed 10 minutes for entire pipetting.

Sample Collection and Preparation:

- The assay can be performed directly on human serum or plasma. But highly lipemic or hemolyzed samples should be discarded.
Materials and Methods

- If the essay is performed within 1-2 days, the sample may be kept at 2-8°C for longer period. It is advisable to freeze sample at -20°C.
- Fibrin filaments may exist in the plasma sample and it could interfere the whole assay. There it is necessary to centrifuge the sample right before testing.

Procedure:

- To prevent any cross contamination among samples, every individual disposable tip should be used for each sample.
- The regents must be warmed up to room temperature of 18-25°C at least for 30 minutes prior to use.
- Shake sample carefully prior to use. If the sample is frozen it must homogenized prior to use.

Method:

- Prepare and label the coated tubes for slandered in duplicate per concentration control serum in duplicate or more sample in single.
- Pipette 25ul of standards, control serum and sample (s) in to each different coated tube.
- Add carefully 100of $^{125}$I tracer in to all coated tube.
- Mix thoroughly and cover the tube with laboratory film or aluminum foil.
- Shake in 300rpm at room temperature (18-25°C) for 45min.
- Aspirate all liquid from the tubes.
- The tube was washed 2 times with 2 ml of distilled water per tube.
Materials and Methods

- Count radioactivity of the tubes for 1 minute by gamma counter. To ensure the validity of $^{125}$I tracer, count the total radioactivity in the plain test tube filled with 100ul of $^{125}$I tracer.

Summary of Procedure:

<table>
<thead>
<tr>
<th>Standard control Sample(s)</th>
<th>50ul</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{125}$I Tracer</td>
<td>50ul</td>
</tr>
<tr>
<td>45min, R. T (18-25°C), 300rpm</td>
<td></td>
</tr>
<tr>
<td>2ml x 2 times Aspiration and Washing (Distilled Water)</td>
<td></td>
</tr>
<tr>
<td>Count the radioactivity in tube for 1min</td>
<td></td>
</tr>
</tbody>
</table>

The whole assay procedures from 1) to 7) can be automated by BRIORIA

**Note:** For reassaying sample with concentrations greater than 200 mlU/ml, dilute the sample an additional 1:5 or 1:10 using the saline multiply by the dilution factor to obtain concentration of the sample.

**Interpretation of the Results:**

Count the radioactivity of the standard, control serum samples and calculate their binding percent. $B/B_{max} (%) = \frac{\text{Solidphasecpm}}{\text{Standard 200(mlU/ml) cpmx100 (})}.\%

**Use Precautions**

- Wear disposable gloves while handling the kit reagents and wash handsthroughly after use.
- Do not smoke, eat or drink in areas where specimens or kit reagents are handled and do not pipette by mouth.
Materials and Methods

- Handle samples, reagents, and laboratory equipments used for assay with extreme care, as they may potentially contain infectious agents.
- Avoid microbial contamination when the reagent vial be eventually opened or the contents be handled.

DATA ANALYSIS (STATISTICAL)

The data is analyzed using ANOVA followed by Tukey's Multiple Comparison Test. The p<0.05 are considered as significant.
3. OBSERVATIONS

Table- 8

Observations- No of Follicles in all 5 Groups

<table>
<thead>
<tr>
<th>Control Group</th>
<th>SB Group</th>
<th>SCF Group</th>
<th>KB Group</th>
<th>KCF Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>31</td>
<td>76</td>
<td>39</td>
<td>78</td>
<td>51</td>
</tr>
<tr>
<td>19</td>
<td>61</td>
<td>61</td>
<td>35</td>
<td>44</td>
</tr>
<tr>
<td>40</td>
<td>77</td>
<td>51</td>
<td>68</td>
<td>80</td>
</tr>
<tr>
<td>21</td>
<td>16</td>
<td>18</td>
<td>20</td>
<td>56</td>
</tr>
<tr>
<td>33</td>
<td>38</td>
<td>28</td>
<td>32</td>
<td>48</td>
</tr>
<tr>
<td>30</td>
<td>65</td>
<td>33</td>
<td>39</td>
<td>42</td>
</tr>
</tbody>
</table>

SB- Swarna Bhasma, SCF- Swarna Containing Compound Formulation i.e. FALAA GOLD, KB- Kaseesa Bhasma and KCF- Kaseesa Containing Compound Formulation i.e. Rajapravrthini Vati.

No of follicles in each group observed were as follows. They are represented as Minimum & Maximum range respectively. Control group: 19-40, SB Group: 16 – 77, SCF Group: 18 – 61, KB Group: 20 – 80 and KCF Group: 42 -80
### Table- 9

Observations- Size of Largest Follicle in mm in all 5 Groups

<table>
<thead>
<tr>
<th>Control Group</th>
<th>SB Group</th>
<th>SCF Group</th>
<th>KB Group</th>
<th>KCF Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3</td>
<td>0.3</td>
<td>0.4</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>0.25</td>
<td>0.3</td>
<td>0.25</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td><strong>0.35</strong></td>
<td><strong>0.2</strong></td>
<td><strong>0.35</strong></td>
<td><strong>0.35</strong></td>
<td><strong>0.2</strong></td>
</tr>
<tr>
<td>0.3</td>
<td><strong>0.4</strong></td>
<td>0.2</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>0.35</td>
<td>0.3</td>
<td>0.25</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td><strong>0.2</strong></td>
<td><strong>0.2</strong></td>
<td>0.35</td>
<td>0.25</td>
<td>0.3</td>
</tr>
</tbody>
</table>

**Size of Largest Follicle** in mm in each group observed were as follows. They are represented as Minimum & Maximum range respectively.

Control group: 0.2 – 0.35, SB Group: 0.2 – 0.4, SCF Group: 0.2 – 0.4, KB Group: 0.2 – 0.35 and KCF Group: 0.2 - 0.3

### Table- 10

Observations- Thickness of Endometrial in mm all 5 Groups

<table>
<thead>
<tr>
<th>Control Group</th>
<th>SB Group</th>
<th>SCF Group</th>
<th>KB Group</th>
<th>KCF Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3</td>
<td>0.7</td>
<td>0.7</td>
<td><strong>0.4</strong></td>
<td>0.8</td>
</tr>
<tr>
<td>0.5</td>
<td>0.7</td>
<td><strong>0.7</strong></td>
<td>0.7</td>
<td>0.8</td>
</tr>
<tr>
<td><strong>0.6</strong></td>
<td><strong>0.4</strong></td>
<td>0.5</td>
<td><strong>0.8</strong></td>
<td>0.5</td>
</tr>
<tr>
<td>0.5</td>
<td><strong>0.8</strong></td>
<td><strong>0.4</strong></td>
<td>0.6</td>
<td><strong>1.1</strong></td>
</tr>
<tr>
<td>0.4</td>
<td>0.8</td>
<td>0.5</td>
<td>0.5</td>
<td><strong>0.3</strong></td>
</tr>
<tr>
<td><strong>0.3</strong></td>
<td>0.5</td>
<td>0.6</td>
<td>0.7</td>
<td>0.7</td>
</tr>
</tbody>
</table>

**Thickness of Endometrial in mm** in each group observed were as follows. They are represented as Minimum & Maximum range respectively. Control group: 0.3 – 0.6, SB Group: 0.4 - 0.8, SCF Group: 0.2 - 0.4, KB Group: 0.4 – 0.8 and KCF Group: 0.3 – 1.1
Table- 11
Observations- Diameter of Uterus in mm in all 5 Groups

<table>
<thead>
<tr>
<th>Control Group</th>
<th>SB Group</th>
<th>SCF Group</th>
<th>KB Group</th>
<th>KCF Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.8</td>
<td>4</td>
<td>4</td>
<td>4.3</td>
<td>4.3</td>
</tr>
<tr>
<td>3.5</td>
<td>3.5</td>
<td>3.9</td>
<td>4.2</td>
<td>4.1</td>
</tr>
<tr>
<td>4.2</td>
<td>4.2</td>
<td>4.2</td>
<td>4.3</td>
<td>3.9</td>
</tr>
<tr>
<td>5</td>
<td>3.8</td>
<td>3.2</td>
<td>3.5</td>
<td>4.2</td>
</tr>
<tr>
<td>4.1</td>
<td>3.7</td>
<td>3.8</td>
<td>3.9</td>
<td>4</td>
</tr>
<tr>
<td>4.5</td>
<td>4</td>
<td>4.2</td>
<td>3.6</td>
<td>4.1</td>
</tr>
</tbody>
</table>

Diameter of Uterus in mm in each group observed were as follows. They are represented as Minimum & Maximum range respectively. Control group: 2.8 – 5, SB Group: 3.5 – 4.2, SCF Group: 3.2 –4.2, KB Group: 3.5 – 4.3 and KCF Group: 3.9 – 4.3

Table- 12
Observations- FSH Levels in all 5 Groups

<table>
<thead>
<tr>
<th>Control Group</th>
<th>SB Group</th>
<th>SCF Group</th>
<th>KB Group</th>
<th>KCF Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.1</td>
<td>0.24</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>0.9</td>
<td>0.16</td>
<td>0.26</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>0.5</td>
<td>0.5</td>
<td>1.4</td>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td>0.7</td>
<td>1</td>
<td>0.5</td>
<td>1.4</td>
<td>1.2</td>
</tr>
<tr>
<td>0.7</td>
<td>1.1</td>
<td>0.4</td>
<td>1.2</td>
<td>1.1</td>
</tr>
<tr>
<td>0.7</td>
<td>1</td>
<td>0.5</td>
<td>1</td>
<td>1.4</td>
</tr>
</tbody>
</table>

FSH Levels in each group observed were as follows. They are represented as Minimum & Maximum range respectively. Control group: 0.5 – 0.9, SB Group: 0.1 – 1.1, SCF Group: 0.24 – 1.4, KB Group: 0.3 – 1.4 and KCF Group: 0.1 – 1.4
Observations

Observations- LH Levels in all 5 Groups

<table>
<thead>
<tr>
<th>Control Group</th>
<th>SB Group</th>
<th>SCF Group</th>
<th>KB Group</th>
<th>KCF Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.6</td>
<td>0.12</td>
<td>1.8</td>
<td>0.8</td>
<td>2.3</td>
</tr>
<tr>
<td>1.1</td>
<td>0.24</td>
<td>2.1</td>
<td>0.5</td>
<td>1.7</td>
</tr>
<tr>
<td>2</td>
<td>0.5</td>
<td>0.4</td>
<td>1.1</td>
<td>1.7</td>
</tr>
<tr>
<td>0.5</td>
<td>2.1</td>
<td>0.3</td>
<td>2</td>
<td>0.9</td>
</tr>
<tr>
<td>0.3</td>
<td>2</td>
<td>0.2</td>
<td>2</td>
<td>0.23</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>0.2</td>
<td>2</td>
<td>0.23</td>
</tr>
</tbody>
</table>

**LH Levels** in each group observed were as follows. They are represented as Minimum & Maximum range respectively. Control group: 0.3 – 1.1, SB Group: 0.12 – 2.1, SCF Group: 0.2 – 2.1, KB Group: 0.5 – 2 and KCF Group: 0.23 – 2.3

Observations- Before Drug Administration Weight of Animals in Grams in all 5 Groups

<table>
<thead>
<tr>
<th>Control Group</th>
<th>SB Group</th>
<th>SCF Group</th>
<th>KB Group</th>
<th>KCF Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>27</td>
<td>23</td>
<td>25</td>
<td>27</td>
</tr>
<tr>
<td>20</td>
<td>32</td>
<td>26</td>
<td>28</td>
<td>23</td>
</tr>
<tr>
<td>15</td>
<td>24</td>
<td>28</td>
<td>26</td>
<td>23</td>
</tr>
<tr>
<td>20</td>
<td>28</td>
<td>30</td>
<td>30</td>
<td>28</td>
</tr>
<tr>
<td>21</td>
<td>31</td>
<td>32</td>
<td>32</td>
<td>29</td>
</tr>
<tr>
<td>20</td>
<td>28</td>
<td>31</td>
<td>29</td>
<td>30</td>
</tr>
</tbody>
</table>

Before Drug Administration Weight of Animals in Grams in each group observed were as follows. They are represented as Minimum & Maximum range respectively. Control group: 15 – 21, SB Group: 27 – 31, SCF Group: 23 – 32, KB Group: 25 – 32 and KCF Group: 23 – 30
Observations- After Drug Administration Weight of Animals in Grams In all 5 Groups

<table>
<thead>
<tr>
<th>Control Group</th>
<th>SB Group</th>
<th>SCF Group</th>
<th>KB Group</th>
<th>KCF Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>21</td>
<td>18</td>
<td>17</td>
<td>18</td>
</tr>
<tr>
<td>21</td>
<td>19</td>
<td>18</td>
<td>18</td>
<td>17</td>
</tr>
<tr>
<td>17</td>
<td>18</td>
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<td>19</td>
<td>19</td>
</tr>
<tr>
<td>20</td>
<td>17</td>
<td>20</td>
<td>18</td>
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</tr>
<tr>
<td>18</td>
<td>20</td>
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<tr>
<td>19</td>
<td>20</td>
<td>20</td>
<td>18</td>
<td>19</td>
</tr>
</tbody>
</table>

After Drug Administration Weight of Animals in Grams in each group observed were as follows. They are represented as Minimum & Maximum range respectively. Control group: 17 – 21, SB Group: 17 – 21, SCF Group: 18 – 21, KB Group: 17 – 20 and KCF Group: 17 – 20

Observations - Weight of Uterus in mg in all 5 Groups

<table>
<thead>
<tr>
<th>Control Group</th>
<th>SB Group</th>
<th>SCF Group</th>
<th>KB Group</th>
<th>KCF Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.08</td>
<td>1.21</td>
<td>0.57</td>
<td>0.47</td>
<td>0.51</td>
</tr>
<tr>
<td>0.12</td>
<td>1.7</td>
<td>0.55</td>
<td>0.44</td>
<td>0.64</td>
</tr>
<tr>
<td>0.12</td>
<td>1.73</td>
<td>0.45</td>
<td>0.71</td>
<td>0.81</td>
</tr>
<tr>
<td>0.09</td>
<td>1.8</td>
<td>0.14</td>
<td>0.81</td>
<td>0.93</td>
</tr>
<tr>
<td>0.13</td>
<td>1.78</td>
<td>0.15</td>
<td>0.91</td>
<td>0.96</td>
</tr>
<tr>
<td>0.8</td>
<td>1.67</td>
<td>0.1</td>
<td>0.96</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Weight of Uterus in mg in each group observed were as follows. They are represented as Minimum & Maximum range respectively. Control group: 0.08 – 0.13, SB Group: 1.8 – 1.21, SCF Group: 0.1 – 0.57, KB Group: 0.44 – 0.96 and KCF Group: 0.51 – 0.98

Vaginal cornification
As animals selected were immature, vagina did not found open in all 5 groups. So, vaginal cornification test was not performed.
4. RESULTS

Table -17

Result of bhasma parikshas of three samples of Swarna Bhasma

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>Champaka Pushpa like yellow</td>
<td>Slightly light brown</td>
<td>Reddish</td>
</tr>
<tr>
<td>Taste</td>
<td>Tasteless</td>
<td>Slight Metallic taste</td>
<td>Tasteless</td>
</tr>
<tr>
<td>Odour</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Touch</td>
<td>Smooth</td>
<td>Smooth</td>
<td>Fine</td>
</tr>
<tr>
<td>Varitara</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Rekhapurna</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Unama</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Apunarbhava</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Niruttha</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>SukshmaandSlakshna</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Nirdhuma</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Nishchandra</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
</tbody>
</table>
Table -18
NPST Results of three samples of Swarna Bhasma

<table>
<thead>
<tr>
<th>Observation</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample3</th>
</tr>
</thead>
<tbody>
<tr>
<td>I phase (0-5 min)</td>
<td>Deep brick red central solid spot with deep brick red margin.</td>
<td>Deep brick red central solid spot with deep brick red margin.</td>
<td>Deep brick red central solid spot with deep brick red margin.</td>
</tr>
<tr>
<td>II Phase (5-20min)</td>
<td>Wet periphery faded with reduction in the brightness.</td>
<td>Wet periphery faded with reduction in the brightness.</td>
<td>Wet periphery faded with reduction in the brightness.</td>
</tr>
<tr>
<td>III Phase (20 min-1day)</td>
<td>Red spot faded with reduction in the brightness.</td>
<td><strong>Brown colour circular spreading with reduction in the brightness</strong></td>
<td><strong>Brown colour circular spreading with reduction in the brightness</strong></td>
</tr>
</tbody>
</table>
### Results of Bhasma Pariksha of three Samples of Kaseesa Bhasma

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>Dark brown</td>
<td>Slightly light brown</td>
<td>Reddish</td>
</tr>
<tr>
<td>Taste</td>
<td>Tasteless</td>
<td>Slight sour taste</td>
<td>Tasteless</td>
</tr>
<tr>
<td>Odour</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Touch</td>
<td>Smooth</td>
<td>Smooth</td>
<td>Fine</td>
</tr>
<tr>
<td>Varitara</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Rekhapurna</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Unama</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Apunarbhava</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Niruttha</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Sukshma and Slakshna</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Nirdhuma</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
</tr>
</tbody>
</table>
### Results

#### Table -20

**NPST Results of three samples of Kaseesa Bhasma**

<table>
<thead>
<tr>
<th>Observation</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>I phase (0-5 min)</td>
<td>Wet periphery forms followed by a thick blue circle in the centre of the spot. But wet spot was not much wide.</td>
<td>Wet periphery appears followed by light blue stain in the centre.</td>
<td>Wet periphery appears followed by light blue circle in the centre.</td>
</tr>
<tr>
<td>II Phase (5-20 min)</td>
<td>Wet periphery faded with reduction in the brightness of blue circle.</td>
<td>Wet periphery became light blue at the end of II phase.</td>
<td>Wet periphery faded away in this phase.</td>
</tr>
<tr>
<td>III Phase (20 min-1 day)</td>
<td>Dark blue central circle.</td>
<td>Light blue centre with light brown periphery</td>
<td>Dark blue center with light blue periphery</td>
</tr>
</tbody>
</table>
### Table -21

**Results of ICPAES Analysis of all 4 Samples**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration of elements</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Au%</td>
</tr>
<tr>
<td>Swarna Bhasma</td>
<td>99.47</td>
</tr>
<tr>
<td>Kaseesa Bhasma</td>
<td></td>
</tr>
<tr>
<td>Falaa Gold (Swarna Bhasma Containing Compound Formulation)</td>
<td>0.00013</td>
</tr>
<tr>
<td>Rajapravartini Vati (Kaseesa Bhasma Containing Compound Formulation)</td>
<td></td>
</tr>
</tbody>
</table>
Results

FTIR RESULTS

**Graph -1 FTIR Report of Swarna Bhasma**

![Swarna Bhasma FTIR Graph](image1)

**Graph -2 FTIR Report of FalaaGold**

![FalaaGold FTIR Graph](image2)
Results

Graph -3 FTIR Report of Kaseesa Bhasma

Graph -4 FTIR Report of Raja Pravartini Vati
Results

FTIR RESULTS

Swarna Bhasma
In Swarna Bhasma OH group with intra molecular H bond was observed at wave number 3427.92 cm\(^{-1}\). CH stretching absent in Swarna Bhasma. C-H bond bending seen at wave number 1380 cm\(^{-1}\). C-C stretching is observed at wave number 1637 cm\(^{-1}\). Halogen observed at wave number 547.11 cm\(^{-1}\) and ether at wave number 1140.29 cm\(^{-1}\).

FALAA Gold
In FALAA Gold OH group with intra molecular H bond was observed at wave number 3405.84 cm\(^{-1}\). CH Stretching wave number 2927.05 cm\(^{-1}\). C-H bond bending seen at wave number 1455.01 cm\(^{-1}\). C-C stretching is observed at wave number 1641.82 cm\(^{-1}\). Halogen observed at wave number 458.07 cm\(^{-1}\). C-O stretching at wave number 1019.85 cm\(^{-1}\) marks with primary alcohol. C-C bending with wave number 670.58 cm\(^{-1}\).

Kaseesa Bhasma
In Kaseesa Bhasma the wave number ranges from 3181.43 cm\(^{-1}\) and 1632.23 cm\(^{-1}\) show the presence of broaden chelate compound and C-C stretching respectively. C=O stretching in Kaseesa bhasma observed at 1632.23 cm\(^{-1}\). Presence of di-substituted marked at 994 cm\(^{-1}\) wave number. Presence of bromine or iodine at 540.07 cm\(^{-1}\).

Rajapravartini Vati
NH group presence is observed through 3403.22 cm\(^{-1}\) wave number. CH stretching in Rajapravartini Vati at 2931.63 cm\(^{-1}\). C=O stretching observed at wave number 1633.03 cm\(^{-1}\) distributed observed at 853.58 cm\(^{-1}\) and 667.09 cm\(^{-1}\). In Raja Pravartini Vati presence of bromine and iodine better separated being 599.84 cm\(^{-1}\) and 465.58 cm\(^{-1}\) respectively. Trans group of halogen may be present.
Results

Graph -5 Swarna Bhasma XRD Results

Graph -6 Swarna Compound Formulation Falaa Gold XRD Results
Results

Graph -7 Kaseesa Bhasma XRD Results

Graph -8 Kaseesa Compound Formulation Rajapravartini Vati XRD Results
Results

XRD Results:

Swarna Bhasma

Crystalline gold with face centered cubic structure (cubic closed packed) were noted in Swarna Bhasma. In Swarna Bhasma the miller indices for silver is noted. For calcium face centered cubic structure. For magnesium hexagonal closed packed. For sodium and potassium body centered cubic structure. For silica diamond cubic structure.

FALAA Gold

Typical pattern of Falaa Gold exhibited different peaks. This indicates crystalline gold with face centered cubic structure (cubic closed packed). Other peaks may be after slight changes in structure due to impurities present or other metals present with Falaa Gold.

Kaseesa Bhasma

Kaseesa Bhasmas Show presence of Iron with body centered cubic structure.

Rajah Pravartini Vati

Rajah Pravartini Vati showed presence of iron with body centered cubic structure. The peaks associated with boron observed in Rajah Pravartini Vati indicating the Rhomboderal shapes. Rajah Pravartini Vati also revealed for sodium and potassium. These all indicate body centered cubic structure. For silica show diamond cubic structure.
Results

Photo - 1 SEM Report of Swarna Bhasma:

- 15kV X1,500 10μm 0000 11 45 SEI
- 15kV X3,000 5μm 0000 11 45 SEI
- 15kV X10,000 1μm 0000 11 45 SEI
- 15kV X6,000 2μm 0000 11 45 SEI
Results

Photo-2 SEM Report of FALAA Gold:
Photo -3 SEM Report of Kasisa Bhasma:
Results

Photo- 4 SEM Report of Rajah Pravartini Vati:
Results

SEM Results:

**Swarna Bhasma:** In Swarna Bhasma, particles of different shapes and sizes are observed adhered to each other in agglutinated mass as well as some of individual particles cubic shapes and hexagonal shapes are seen. Adherence of particles gives the appearance of octahedral shape too. The size varies from 0.5µm to 20 µm.

**Falaa Gold:** SEM analysis of Falaa Gold shows the particles of face-centered cubic structures adhered with each other and separated as well. The particle size varies from 0.08 µm to 15 µm.

**Kaseesa Bhasma:** Kaseesa Bhasma shows minute particles of different shapes (mostly cubic). The size varies from 0.01µm to 15 µm. Some of particles are even smaller than 0.01µm.

**Rajaha Pravaratini Vati:** In Rajaha Pravaratini Vati, particles are seen with more lustered. Shapes and size also varies widely. Some of particles are seen even with pointed heads while common one is cubic shapes, usually packed in nature. Rhombohedral, hexahedral and diamond-shaped particles are also seen in abundance. The size varies from 0.05µm (even smaller) to greater than 30 µm.
### Table -22

**Statistical Analysis of no of Follicles in all 5 Groups**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control Group</th>
<th>SB Group</th>
<th>SCF Group</th>
<th>KB Group</th>
<th>KCF Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>29.00±7.82</td>
<td>55.50±23.96</td>
<td>38.33±15.64</td>
<td>45.33±22.57</td>
<td>53.50±13.91</td>
</tr>
<tr>
<td>p value</td>
<td>0.0891</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Note:**
- SB - Swarna Bhasma
- KB - Kaseesa Bhasma

SCF-Swarna Bhasma Compound Formulation i.e FALAA GOLD.

KCF-Kaseesa Bhasma Compound Formulation i.e RajapravrthiniVati.

### Table -23

**Comparative Statistical Analysis between the Groups w.r.t no of Follicles in all 5 Groups**

<table>
<thead>
<tr>
<th>Tukey's Multiple Comparison Test</th>
<th>Mean Diff.</th>
<th>Q</th>
<th>Significant P &lt; 0.05</th>
<th>Summary</th>
<th>95% CI of diff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control vs SB</td>
<td>-26.50</td>
<td>3.648</td>
<td>No</td>
<td>Not Significant</td>
<td>-56.68 to 3.682</td>
</tr>
<tr>
<td>Control vs SCF</td>
<td>-9.333</td>
<td>1.285</td>
<td>No</td>
<td>Not Significant</td>
<td>-39.52 to 20.85</td>
</tr>
<tr>
<td>Control vs KB</td>
<td>-16.33</td>
<td>2.249</td>
<td>No</td>
<td>Not Significant</td>
<td>-46.52 to 13.85</td>
</tr>
<tr>
<td>Control vs KCF</td>
<td>-24.50</td>
<td>3.373</td>
<td>No</td>
<td>Not Significant</td>
<td>-54.68 to 5.682</td>
</tr>
<tr>
<td>SB vs SCF</td>
<td>17.17</td>
<td>2.363</td>
<td>No</td>
<td>Not Significant</td>
<td>-13.02 to 47.35</td>
</tr>
<tr>
<td>SB vs KB</td>
<td>10.17</td>
<td>1.400</td>
<td>No</td>
<td>Not Significant</td>
<td>-20.02 to 40.35</td>
</tr>
<tr>
<td>SB vs KCF</td>
<td>2.000</td>
<td>0.2754</td>
<td>No</td>
<td>Not Significant</td>
<td>-28.18 to 32.18</td>
</tr>
<tr>
<td>SCF vs KB</td>
<td>-7.000</td>
<td>0.9637</td>
<td>No</td>
<td>Not Significant</td>
<td>-37.18 to 23.18</td>
</tr>
<tr>
<td>SCF vs KCF</td>
<td>-15.17</td>
<td>2.088</td>
<td>No</td>
<td>Not Significant</td>
<td>-45.35 to 15.02</td>
</tr>
<tr>
<td>KB vs KCF</td>
<td>-8.167</td>
<td>1.124</td>
<td>No</td>
<td>Not Significant</td>
<td>-38.35 to 22.02</td>
</tr>
</tbody>
</table>
Table -24
Statistical Analysis of Size of Largest Follicle in all 5 Groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control Group</th>
<th>SB Group</th>
<th>SCF Group</th>
<th>KB Group</th>
<th>KCF Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ±SD</td>
<td>0.29 ± 0.05</td>
<td>0.28 ± 0.07</td>
<td>0.30 ± 0.07</td>
<td>0.28 ± 0.05</td>
<td>0.28 ± 0.04</td>
</tr>
<tr>
<td>p value</td>
<td>0.9860</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table -25
Comparative Statistical Analysis between the Groups w.r.t Size of Largest Follicle in all 5 Groups

<table>
<thead>
<tr>
<th>Tukey's Multiple Comparison Test</th>
<th>Mean Diff.</th>
<th>Q</th>
<th>Significant P &lt; 0.05</th>
<th>Summary</th>
<th>95% CI of diff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control vs SB</td>
<td>0.0083</td>
<td>0.3276</td>
<td>No</td>
<td>Not Significant</td>
<td>-0.09738 to 0.1140</td>
</tr>
<tr>
<td>Control vs SCF</td>
<td>-0.008</td>
<td>0.3276</td>
<td>No</td>
<td>Not Significant</td>
<td>-0.1140 to 0.09738</td>
</tr>
<tr>
<td>Control vs KB</td>
<td>0.008</td>
<td>0.3276</td>
<td>No</td>
<td>Not Significant</td>
<td>-0.09738 to 0.1140</td>
</tr>
<tr>
<td>Control vs KCF</td>
<td>0.008</td>
<td>0.3276</td>
<td>No</td>
<td>Not Significant</td>
<td>-0.09738 to 0.1140</td>
</tr>
<tr>
<td>SB vs SCF</td>
<td>-0.01</td>
<td>0.6551</td>
<td>No</td>
<td>Not Significant</td>
<td>-0.1224 to 0.08905</td>
</tr>
<tr>
<td>SB vs KB</td>
<td>0.00</td>
<td>0.0000</td>
<td>No</td>
<td>Not Significant</td>
<td>-0.1057 to 0.1057</td>
</tr>
<tr>
<td>SB vs KCF</td>
<td>0.00</td>
<td>0.0000</td>
<td>No</td>
<td>Not Significant</td>
<td>-0.1057 to 0.1057</td>
</tr>
<tr>
<td>SCF vs KB</td>
<td>0.016</td>
<td>0.6551</td>
<td>No</td>
<td>Not Significant</td>
<td>-0.08905 to 0.1224</td>
</tr>
<tr>
<td>SCF vs KCF</td>
<td>0.016</td>
<td>0.6551</td>
<td>No</td>
<td>Not Significant</td>
<td>-0.08905 to 0.1224</td>
</tr>
<tr>
<td>KB vs KCF</td>
<td>0.00</td>
<td>0.0000</td>
<td>No</td>
<td>Not Significant</td>
<td>-0.1057 to 0.1057</td>
</tr>
</tbody>
</table>
Table -26
Statistical Analysis of thickness of endometrium in all 5 Groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control Group</th>
<th>SB Group</th>
<th>SCF Group</th>
<th>KB Group</th>
<th>KCF Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>0.43 ± 0.12</td>
<td>0.65 ± 0.16</td>
<td>0.57 ± 0.12</td>
<td>0.62 ± 0.15</td>
<td>0.70 ± 0.28</td>
</tr>
<tr>
<td>p value</td>
<td>0.1226</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table -27
Comparative Statistical Analysis between the Groups w.r.t Thickness of Endometrium in all 5 Groups

<table>
<thead>
<tr>
<th>Tukey's Multiple Comparison Test</th>
<th>Mean Diff.</th>
<th>Q</th>
<th>Significant P &lt; 0.05</th>
<th>Summary</th>
<th>95% CI of diff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control vs SB</td>
<td>-0.22</td>
<td>3.0</td>
<td>No</td>
<td>Not Significant</td>
<td>-0.51 to 0.081</td>
</tr>
<tr>
<td>Control vs SCF</td>
<td>-0.13</td>
<td>1.9</td>
<td>No</td>
<td>Not Significant</td>
<td>-0.43 to 0.16</td>
</tr>
<tr>
<td>Control vs KB</td>
<td>-0.18</td>
<td>2.6</td>
<td>No</td>
<td>Not Significant</td>
<td>-0.48 to 0.11</td>
</tr>
<tr>
<td>Control vs KCF</td>
<td>-0.27</td>
<td>3.7</td>
<td>No</td>
<td>Not Significant</td>
<td>-0.56 to 0.031</td>
</tr>
<tr>
<td>SB vs SCF</td>
<td>0.083</td>
<td>1.2</td>
<td>No</td>
<td>Not Significant</td>
<td>-0.21 to 0.38</td>
</tr>
<tr>
<td>SB vs KB</td>
<td>0.033</td>
<td>0.47</td>
<td>No</td>
<td>Not Significant</td>
<td>-0.26 to 0.33</td>
</tr>
<tr>
<td>SB vs KCF</td>
<td>-0.050</td>
<td>0.70</td>
<td>No</td>
<td>Not Significant</td>
<td>-0.35 to 0.25</td>
</tr>
<tr>
<td>SCF vs KB</td>
<td>-0.050</td>
<td>0.70</td>
<td>No</td>
<td>Not Significant</td>
<td>-0.35 to 0.25</td>
</tr>
<tr>
<td>SCF vs KCF</td>
<td>-0.13</td>
<td>1.9</td>
<td>No</td>
<td>Not Significant</td>
<td>-0.43 to 0.16</td>
</tr>
<tr>
<td>KB vs KCF</td>
<td>-0.083</td>
<td>1.2</td>
<td>No</td>
<td>Not Significant</td>
<td>-0.38 to 0.21</td>
</tr>
</tbody>
</table>
Results

Table -28
Statistical Analysis of diameter of uterus in all 5 Groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control Group</th>
<th>SB Group</th>
<th>SCF Group</th>
<th>KB Group</th>
<th>KCF Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>4.0 ± 0.77</td>
<td>3.9 ± 0.25</td>
<td>3.9 ± 0.37</td>
<td>4.0 ± 0.36</td>
<td>4.1 ± 0.14</td>
</tr>
<tr>
<td>p value</td>
<td>0.8781</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table -29
Comparative Statistical analysis between the groups w.r.t diameter of uterus in all 5 Groups

<table>
<thead>
<tr>
<th>Tukey's Multiple Comparison Test</th>
<th>Mean Diff.</th>
<th>Q</th>
<th>Significant</th>
<th>Summary</th>
<th>95% CI of diff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control vs SB</td>
<td>0.15</td>
<td>0.85</td>
<td>No</td>
<td>Not Significant</td>
<td>-0.59 to 0.89</td>
</tr>
<tr>
<td>Control vs SCF</td>
<td>0.13</td>
<td>0.75</td>
<td>No</td>
<td>Not Significant</td>
<td>-0.60 to 0.87</td>
</tr>
<tr>
<td>Control vs KB</td>
<td>0.050</td>
<td>0.28</td>
<td>No</td>
<td>Not Significant</td>
<td>-0.69 to 0.79</td>
</tr>
<tr>
<td>Control vs KCF</td>
<td>-0.083</td>
<td>0.47</td>
<td>No</td>
<td>Not Significant</td>
<td>-0.82 to 0.65</td>
</tr>
<tr>
<td>SB vs SCF</td>
<td>-0.017</td>
<td>0.094</td>
<td>No</td>
<td>Not Significant</td>
<td>-0.75 to 0.72</td>
</tr>
<tr>
<td>SB vs KB</td>
<td>-0.10</td>
<td>0.56</td>
<td>No</td>
<td>Not Significant</td>
<td>-0.84 to 0.64</td>
</tr>
<tr>
<td>SB vs KCF</td>
<td>-0.23</td>
<td>1.3</td>
<td>No</td>
<td>Not Significant</td>
<td>-0.97 to 0.50</td>
</tr>
<tr>
<td>SCF vs KB</td>
<td>-0.083</td>
<td>0.47</td>
<td>No</td>
<td>Not Significant</td>
<td>-0.82 to 0.65</td>
</tr>
<tr>
<td>SCF vs KCF</td>
<td>-0.22</td>
<td>1.2</td>
<td>No</td>
<td>Not Significant</td>
<td>-0.95 to 0.52</td>
</tr>
<tr>
<td>KB vs KCF</td>
<td>-0.13</td>
<td>0.75</td>
<td>No</td>
<td>Not Significant</td>
<td>-0.87 to 0.60</td>
</tr>
</tbody>
</table>
Table -30
Statistical Analysis of FSH levels in all 5 Groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control Group</th>
<th>SB Group</th>
<th>SCF Group</th>
<th>KB Group</th>
<th>KCF Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>0.67 ± 0.15</td>
<td>0.64 ± 0.45</td>
<td>0.55 ± 0.43</td>
<td>0.77 ± 0.49</td>
<td>0.78 ± 0.53</td>
</tr>
<tr>
<td>p value</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.8787</td>
</tr>
</tbody>
</table>

Table -31
Comparative Statistical Analysis between the Groups w.r.t FSH Levels in all 5 Groups

<table>
<thead>
<tr>
<th>Tukey's Multiple Comparison Test</th>
<th>Mean Diff.</th>
<th>Q</th>
<th>Significant P &lt; 0.05</th>
<th>Summary</th>
<th>95% CI of diff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control vs SB</td>
<td>0.023</td>
<td>0.13</td>
<td>No</td>
<td>Not Significant</td>
<td>-0.71 to 0.76</td>
</tr>
<tr>
<td>Control vs SCF</td>
<td>0.12</td>
<td>0.66</td>
<td>No</td>
<td>Not Significant</td>
<td>-0.62 to 0.85</td>
</tr>
<tr>
<td>Control vs KB</td>
<td>-0.10</td>
<td>0.57</td>
<td>No</td>
<td>Not Significant</td>
<td>-0.83 to 0.63</td>
</tr>
<tr>
<td>Control vs KCF</td>
<td>-0.12</td>
<td>0.66</td>
<td>No</td>
<td>Not Significant</td>
<td>-0.85 to 0.62</td>
</tr>
<tr>
<td>SB vs SCF</td>
<td>0.093</td>
<td>0.53</td>
<td>No</td>
<td>Not Significant</td>
<td>-0.64 to 0.83</td>
</tr>
<tr>
<td>SB vs KB</td>
<td>-0.12</td>
<td>0.70</td>
<td>No</td>
<td>Not Significant</td>
<td>-0.86 to 0.61</td>
</tr>
<tr>
<td>SB vs KCF</td>
<td>-0.14</td>
<td>0.79</td>
<td>No</td>
<td>Not Significant</td>
<td>-0.87 to 0.59</td>
</tr>
<tr>
<td>SCF vs KB</td>
<td>-0.22</td>
<td>1.2</td>
<td>No</td>
<td>Not Significant</td>
<td>-0.95 to 0.52</td>
</tr>
<tr>
<td>SCF vs KCF</td>
<td>-0.23</td>
<td>1.3</td>
<td>No</td>
<td>Not Significant</td>
<td>-0.97 to 0.50</td>
</tr>
<tr>
<td>KB vs KCF</td>
<td>-0.017</td>
<td>0.095</td>
<td>No</td>
<td>Not Significant</td>
<td>-0.75 to 0.72</td>
</tr>
</tbody>
</table>
### Table -32
Statistical Analysis of LH levels in all 5 Groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control Group</th>
<th>SB Group</th>
<th>SCF Group</th>
<th>KB Group</th>
<th>KCF Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>0.92 ± 0.61</td>
<td>1.2 ± 0.97</td>
<td>1.2 ± 0.88</td>
<td>1.4 ± 0.65</td>
<td>1.2 ± 0.86</td>
</tr>
<tr>
<td>p value</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.9107</td>
</tr>
</tbody>
</table>

### Table -33
Comparative Statistical Analysis between the Groups w.r.t LH Levels in all 5 Groups

<table>
<thead>
<tr>
<th>Tukey's Multiple Comparison Test</th>
<th>Mean Diff.</th>
<th>Q</th>
<th>Significant p&lt; 0.05</th>
<th>Summary</th>
<th>95% CI of diff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control vs SB</td>
<td>-0.24</td>
<td>0.75</td>
<td>No</td>
<td>Not Significant</td>
<td>-1.6 to 1.1</td>
</tr>
<tr>
<td>Control vs SCF</td>
<td>-0.28</td>
<td>0.78</td>
<td>No</td>
<td>Not Significant</td>
<td>-1.8 to 1.2</td>
</tr>
<tr>
<td>Control vs KB</td>
<td>-0.45</td>
<td>1.4</td>
<td>No</td>
<td>Not Significant</td>
<td>-1.8 to 0.91</td>
</tr>
<tr>
<td>Control vs KCF</td>
<td>-0.26</td>
<td>0.80</td>
<td>No</td>
<td>Not Significant</td>
<td>-1.6 to 1.1</td>
</tr>
<tr>
<td>SB vs SCF</td>
<td>-0.040</td>
<td>0.11</td>
<td>No</td>
<td>Not Significant</td>
<td>-1.6 to 1.5</td>
</tr>
<tr>
<td>SB vs KB</td>
<td>-0.21</td>
<td>0.63</td>
<td>No</td>
<td>Not Significant</td>
<td>-1.6 to 1.2</td>
</tr>
<tr>
<td>SB vs KCF</td>
<td>-0.017</td>
<td>0.051</td>
<td>No</td>
<td>Not Significant</td>
<td>-1.4 to 1.3</td>
</tr>
<tr>
<td>SCF vs KB</td>
<td>-0.17</td>
<td>0.46</td>
<td>No</td>
<td>Not Significant</td>
<td>-1.7 to 1.4</td>
</tr>
<tr>
<td>SCF vs KCF</td>
<td>0.023</td>
<td>0.064</td>
<td>No</td>
<td>Not Significant</td>
<td>-1.5 to 1.5</td>
</tr>
<tr>
<td>KB vs KCF</td>
<td>0.19</td>
<td>0.58</td>
<td>No</td>
<td>Not Significant</td>
<td>-1.2 to 1.6</td>
</tr>
</tbody>
</table>
### Table -34
Statistical Analysis of Weight of Animals in all 5 Groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control Group</th>
<th>SB Group</th>
<th>SCF Group</th>
<th>KB Group</th>
<th>KCF Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>0.17±1.7</td>
<td>9.2±2.9</td>
<td>9.0±2.3</td>
<td>10±2.1</td>
<td>8.2±2.8</td>
</tr>
<tr>
<td>p value</td>
<td>0.0001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table -35
Comparative Statistical Analysis between the Groups w.r.t Weight of Animals in all 5 Groups

<table>
<thead>
<tr>
<th>Tukey's Multiple Comparison Test</th>
<th>Mean Diff.</th>
<th>Q</th>
<th>Significant P &lt; 0.05</th>
<th>Summary</th>
<th>95% CI of diff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control vs SB</td>
<td>-9.0</td>
<td>9.2</td>
<td>Yes</td>
<td>Significant***</td>
<td>-13 to -4.9</td>
</tr>
<tr>
<td>Control vs SCF</td>
<td>-8.8</td>
<td>9.0</td>
<td>Yes</td>
<td>Significant***</td>
<td>-13 to -4.8</td>
</tr>
<tr>
<td>Control vs KB</td>
<td>-9.8</td>
<td>10</td>
<td>Yes</td>
<td>Significant***</td>
<td>-14 to -5.8</td>
</tr>
<tr>
<td>Control vs KCF</td>
<td>-8.0</td>
<td>8.2</td>
<td>Yes</td>
<td>Significant***</td>
<td>-12 to -3.9</td>
</tr>
<tr>
<td>SB vs SCF</td>
<td>0.17</td>
<td>0.17</td>
<td>No</td>
<td>Not Significant</td>
<td>-3.9 to 4.2</td>
</tr>
<tr>
<td>SB vs KB</td>
<td>-0.83</td>
<td>0.85</td>
<td>No</td>
<td>Not Significant</td>
<td>-4.9 to 3.2</td>
</tr>
<tr>
<td>SB vs KCF</td>
<td>1.0</td>
<td>1.0</td>
<td>No</td>
<td>Not Significant</td>
<td>-3.1 to 5.1</td>
</tr>
<tr>
<td>SCF vs KB</td>
<td>-1.0</td>
<td>1.0</td>
<td>No</td>
<td>Not Significant</td>
<td>-5.1 to 3.1</td>
</tr>
<tr>
<td>SCF vs KCF</td>
<td>0.83</td>
<td>0.85</td>
<td>No</td>
<td>Not Significant</td>
<td>-3.2 to 4.9</td>
</tr>
<tr>
<td>KB vs KCF</td>
<td>1.8</td>
<td>1.9</td>
<td>No</td>
<td>Not Significant</td>
<td>-2.2 to 5.9</td>
</tr>
</tbody>
</table>
Table -36
Statistical Analysis of Weight of Uterus in all 5 Groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control Group</th>
<th>SB Group</th>
<th>SCF Group</th>
<th>KB Group</th>
<th>KCF Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ±SD</td>
<td>0.22±0.28</td>
<td>1.6±0.22</td>
<td>0.33±0.22</td>
<td>0.72±0.22</td>
<td>0.81±0.19</td>
</tr>
<tr>
<td>p value</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Table -37
Comparative Statistical Analysis between the Groups w.r.t Weight of Uterus in all 5 Groups

<table>
<thead>
<tr>
<th>Tukey's Multiple Comparison Test</th>
<th>Mean Diff.</th>
<th>Q</th>
<th>Significant P &lt; 0.05</th>
<th>Summary</th>
<th>95% CI of diff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control vs SB</td>
<td>-1.4</td>
<td>15</td>
<td>Yes</td>
<td>Significant***</td>
<td>-1.8 to -1.0</td>
</tr>
<tr>
<td>Control vs SCF</td>
<td>-0.10</td>
<td>1.1</td>
<td>No</td>
<td>Ns</td>
<td>-0.49 to 0.29</td>
</tr>
<tr>
<td>Control vs KB</td>
<td>-0.49</td>
<td>5.3</td>
<td>Yes</td>
<td>Significant**</td>
<td>-0.88 to -0.10</td>
</tr>
<tr>
<td>Control vs KCF</td>
<td>-0.58</td>
<td>6.2</td>
<td>Yes</td>
<td>Significant**</td>
<td>-0.97 to -0.19</td>
</tr>
<tr>
<td>SB vs SCF</td>
<td>1.3</td>
<td>14</td>
<td>Yes</td>
<td>Significant***</td>
<td>0.93 to 1.7</td>
</tr>
<tr>
<td>SB vs KB</td>
<td>0.93</td>
<td>10</td>
<td>Yes</td>
<td>Significant***</td>
<td>0.54 to 1.3</td>
</tr>
<tr>
<td>SB vs KCF</td>
<td>0.84</td>
<td>9.0</td>
<td>Yes</td>
<td>Significant***</td>
<td>0.45 to 1.2</td>
</tr>
<tr>
<td>SCF vs KB</td>
<td>-0.39</td>
<td>4.2</td>
<td>Yes</td>
<td>Significant*</td>
<td>-0.78 to -0.0013</td>
</tr>
<tr>
<td>SCF vs KCF</td>
<td>-0.48</td>
<td>5.1</td>
<td>Yes</td>
<td>Significant*</td>
<td>-0.87 to -0.090</td>
</tr>
<tr>
<td>KB vs KCF</td>
<td>-0.088</td>
<td>0.94</td>
<td>No</td>
<td>Not Significant</td>
<td>-0.48 to 0.30</td>
</tr>
</tbody>
</table>
5. DISCUSSION

Selection of Bhasma

Bhasma Parikshas

3 Samples of Swarna and 3 samples of Kaseesa Bhasma samples were subjected to classical Bhasma Pareeksha, NPST and further elemental analysis to select a good quality bhasma for the study. Classical bhasma pareeksha are adopted for initial selection of both bhasma because bhasma pareeksha explains physical as well as chemical properties of bhasma which are mentioned below:

Physical – Varitaratwa, Rekhapurnatwa, Unam /Uttama test for Bhasma, Sukshma, Slakshna, Laguta (Lightness)

Chemical - Apunarbhava (Not regaining its metallic state), Niruthya, Swadarahita (Tasteless), Nirdhumatwa (not producing fumes on exposure to fire) and Avami (Not causing vomiting)

Among three samples of Swarna Bhasma, sample 1 showed classical bhasma Varna (colour) i.e. champak pushpa, tasteless and passed all relevant Bhasma Parikshas.

Whereas Sample 2 and 3 passed all the bhasma pareeksha except that of colour, taste and Nirdhuma which are very important tests to indicate its physical and chemical nature. Change in colour, taste and not passing Nirdhuma pareeksha are the indications that bhasma may be having Gandhaka etc metals in trace which may be responsible for change in the colour as well as taste. Further all the 3 samples of Swarna Bhasma subjected to Namburi Phased Spot test.

Among three samples of Kaseesa Bhasma, sample1 passed all relevant bhasma parikshas. Whereas Sample 2 did not pass Nirdhuma and sample 3 Varitara, Slakshna
bhasma pareeksha. The reason for not passing Nirdhuma parikshas may be presence of Gandhaka or organic impurities and reason for not passing Varitara test may be bhasma has not converted into micro or nano size which is making the bhasma to sink down by breaking the surface tension of water. Further all samples were subjected to NPST test to access qualitatively. After NPST Sample 1 spot matched with standard NPST result. So, it was selected for the study.

**Inductively Coupled Plasma Atomic Emission Spectroscopy (ICPAES) Analysis of all four samples.**

ICPAES analysis of previous studies conducted by V Yadav et.al\(^6\), \(^7\) reported that SB contains elemental gold as 66.12, 92.19% and Brown CL et.al 92% whereas in the present study it was found 99.47%. Which indicates selected Swarna bhasma was of standard quality. Where as its compound formulation FALAA Gold contains 0.00013% gold.

Kaseesa Bhasma contains 41.66% iron and where as its compound formulation Rajapravartini Vati contains 0.93% of iron.

**Fourier Transform Infrared Spectroscopy (FTIR) Analysis of Swarna Bhasma and Falaa Gold**

The Falaa Gold shows wave number range 3405.84 cm\(^{-1}\) indicating OH group with intra-molecular H bond while this range changes to 3427.92 cm\(^{-1}\)showing OH group with intermolecular H bond in Swarna Bhasma. C-H stretching observed with 2927.05 cm\(^{-1}\) in
Falaa Gold is absent in Swarna Bhasma. Wave number 1707.60 cm\(^{-1}\) of Swarna Bhasma indicates keto group. Energy absorbed due to C-H bond bending shows wave number 1380 cm\(^{-1}\) in Swarna Bhasma which is slightly changed from that observed from Falaa Gold being 1455.01 cm\(^{-1}\). C=C stretching is noted in both Swarna Bhasma and Falaa gold, only wave number found slightly changing from 1641.82 cm\(^{-1}\) to 1637 cm\(^{-1}\). Halogens observed in both cases of Swarna Bhasma and Falaa Gold exhibited changing wave numbers respectively being 547.11 cm\(^{-1}\) and 458.07 cm\(^{-1}\). Wave number indicating ether was present with Swarna Bhasma being 1140.29 cm\(^{-1}\). In Falaa Gold wave number of 1248.15 cm\(^{-1}\) shows C-O stretching while of 1019.85 cm\(^{-1}\) marks C-O stretching with primary alcohol. C-C bending with 670.58 cm\(^{-1}\) wave number is also marked in Falaa gold. FTIR results show Swarna Bhasma and Falaa Gold are having similar groups as well as bonding. Only CH\(^{+}\)stretching absent in Swarna Bhasma, which is present in Falaa Gold.

**Fourier Transform Infrared Spectroscopy (FTIR) Analysis of Kaseesa Bhasma and Raja Pravartini Vati**

The wave number ranges of 3181.43 cm\(^{-1}\) and 1632.23 cm\(^{-1}\) show presence of broaden chelate compound and C=C stretching respectively in Kaseesa Bhasma whereas Raja PravartiniVati reveals presence of NH group through 3403.22 cm\(^{-1}\) wave number. NH group might have come from protein breakdown of used seeds or other parts of component drugs. 2931.63 cm\(^{-1}\) indicating C-H stretching is observed with Rajah Pravartini Vati only while wave number 1633.03 cm\(^{-1}\) marks common C=O stretching in both cases with slight change in energy. Presence of di-substituted can be marked from wave number 994 cm\(^{-1}\) in Kaseesa Bhasma which changes in energy in Raja Pravartini
Discussion

Vati being 853.58 cm\(^{-1}\) and 667.09 cm\(^{-1}\). Wave number 540.07 cm\(^{-1}\) exposes the presence of bromine or iodine in Kaseesa Bhasma which is better separated in Raja Pravartini Vati being 599.84 cm\(^{-1}\) and 465.58 cm\(^{-1}\) in sequence for bromine and iodine. Transposition of halogen groups may be present. Raja Pravartini Vati also shows wave numbers 1378.08 cm\(^{-1}\), 1146.19 cm\(^{-1}\) and 763.68 cm\(^{-1}\) which respectively indicate C-H, presence CN stretching and CH\(_2\) (rocking).

FTIR result show Kaseesa Bhasma as well as its compound formulation Rajah Pravartini Vati are having similar groups as well as bonds. Only Broaden chelate compound which is present in Kaseesa Bhasma is absent in Rajah Pravartini Vati.

**X-ray Diffraction Analysis of Swarna Bhasma and FALAA Gold:**

X-ray diffraction was done by using the standard data JCPDS – KDD and X-Ray Diffraction Crystallography by Waseda Yoshio, Mastubara Elchiro and Shinoda Kozo by Springer Publication. In this the relative intensities and their corresponding a < d values are to be tallied with standard values of compounds. Also It was used for structural characterization. Typical pattern of Falaa Gold exhibited different peaks. The characteristic peaks corresponding to miller indices (h, k, l) 111, 200, 220 are located at 2\(\Theta\) 38.12\(^{\circ}\), 44.43\(^{\circ}\) and 64.69\(^{\circ}\). This indicates crystalline gold with face centered cubic structure (cubic closed packed). Other peaks may be after slight changes in structure due to impurities present or other metals present with Falaa Gold.

Similar patterns were noted in Swarna Bhasma at 2\(\Theta\) 38.20\(^{\circ}\), 44.44\(^{\circ}\) and 64.85\(^{\circ}\). In Swarna Bhasma the miller indices for silver is noted for peaks having 2\(\Theta\) 38.54\(^{\circ}\), 44.68\(^{\circ}\),
Discussion

65.05° and 78.21° as 111, 200, 220 and 311. All of them shows face centered cubic structure (cubic closed packed) structure.

2θ 26.35° and 28.60° show miller indices 111 and 200 respectively for calcium. Its structure is face centered cubic structure (cubic closed packed). For magnesium for 2θ 20.30° miller indices found was 1010. Hence, the structure hexagonal closed packed. For sodium and potassium 2θ 32.69°, 32.76° and 32.93° corresponds to miller indices 002 and 200 only whereas 2θ 45.21° suggests 202. For 2θ 51.29°, the miller indices noted is 103. These all indicate body centered cubic structure. For silica, 2θ 27.05°, 48.05°, 57.04° and 69.16° respectively represent miller indices of 111, 220, 311 and 400. They show diamond cubic structure.

XRD graphs show Crystalline gold with face centered cubic structure (cubic closed packed) in Swarna Bhasma as well as in its compound formulation Falaa Gold.

X-ray Diffraction Analysis of Kaseesa Bhasma and Rajah Pravartini Vati:

In Kaseesa Bhasma, 2θ 30.80° and 43.76 ° show miller indices 220 and 400 respectively for iron. Both of them have body centered cubic structure whereas in Rajaha Pravartini Vati, 2θ values shifts to 30.90° and 43.90 °. The peaks associated with boron observed in Rajah Pravartini Vati are at 2θ angles 26.67° and 55.04° corresponding to reflections from the 0003 and 0006 planes. 2θ angles 21.51° and 33.17° show miller indices 1120 and 1210 respectively indicating the rhomboderal shapes. In presence of redundant index, the indices 1120 and 1210 of four planes exhibit similarities with indices of three planes 110 and 120. Like to Swarna bhasma, Rajaha Pravartini Vati also revealed the 2θ values for sodium and potassium. The values shifted to 32.67°, 32.79° and 33.17° corresponding...
Effect of Swarna Bhasma and Kaseesa Bhasma on Reproductive Function in Female Rats

Discussion

To miller indices 002 and 200. $2\Theta$ 45.23° suggests the miller indices 202 where $2\Theta$ 51.25°
103. These all indicate body centered cubic structure. For silica, $2\Theta$ 27.05°, 48.05°,
57.04° and 69.16° respectively represent miller indices of 111, 220, 311 and 400. They
show diamond cubic structure.

XRD graphs show Kaseesa Bhasma as well as its compound formulation Rajah Pravartini
Vati show presence of Iron with body centered cubic structure.

**Scanning Electron Microscope (SEM) Analysis of Swarna Bhasma and Falaa Gold:** In Swarna Bhasma, particles of different shapes and sizes are observed adhered to
each other in agglutinated mass as well as some of individual particles cubic shapes and
hexagonal shapes are seen. Adherence of particles gives the appearance of octahedral
shape too. The size varies from 0.5µm to 20 µm. SEM analysis of Falaa Gold shows the
particles of face-centered cubic structures adhered with each other and separated as well.
The particle size varies from 0.08 µm to 15 µm.

**Scanning Electron Microscope (SEM) Analysis of Kaseesa Bhasma and Rajah Pravartini Vati:** The SEM analysis of Kaseesa Bhasma shows minute particles of
different shapes (mostly cubic). The size varies from 0.01µm to 15 µm. Previous studies
by Keerthy et.al reported Kaseesa bhasma particle size varies from 7-10 µm. which is
nearer to the particle sizes found in present study. Some of particles are even smaller
than 0.01µm. In Raja Pravaratini Vati, particles are seen with more lustered. Shapes and
size also varies widely. Some of particles are seen even with pointed heads while
common one is cubic shapes, usually packed in nature. Rhombohedral, hexahedral and diamond-shaped particles are also seen in abundance. The size varies from 0.05µm (even smaller) to greater than 30 µm.

Our FTIR, XRD and SEM results are matching with the results of earlier FTIR studies on Swarna Bhasma sample by C.L.Brown et.al and Ujjal Kumar Sur et.al.

The SEM, XRD, FTIR results of Kaseesa Bhasma are also matching with the article published by Ujjal Kumar Sur et.al.

During the 1st open house the expert committee suggested addition of compound formulation along with Swarna Bhasma and Kaseesa Bhasma for if a single bhasma does not show its effect on the reproductive system, it is still possible to explore the effect of the compound formulations of Swarna Bhasma and Kaseesa Bhasma.

**Rajapravartini Vati**

In RajapravarthiniVati Shuddha Kaseesa is used. Usually Kaseesa Shodhana is done with Nimbu Swarasa or Bringaraja Swarasa Bhavana. As per above reference after Shodhana itself most part of the Kaseesa gets converted into ferric Sulphate and ferric oxide. While preparing RajapravarthiniVati again Shuddha Kaseesa comes in contact with tankana which is alkaline in nature, Kanya Swarasa which is liquid media. So, further oxidation takes place and converted into ferric oxide. Though ferrous sulphate is garbhashaya shodhaka where as in the selected compound RajapravarthiniVati ferrous suphate gets converted into ferric oxide which is Garbhashaya Poshaka. Same is justified based on below reference.
In moist air, ferrous sulfate rapidly oxidizes and becomes coated with brownish-yellow ferric sulphate and ferric oxide. The rate of oxidation increases by the addition of alkali or by exposure to light.\textsuperscript{206, 207}

\[
12 \text{FeSO}_4 + 3 \text{O}_2 \rightarrow 4 \text{Fe}_2(\text{SO}_4)_3 + 2 \text{Fe}_2\text{O}_3
\]

When Kaseesa Bhasma alone is showing increase in the uterus weight and no of follicles. In Rajapravartini Vati the quantity of Kaseesa is less. Therefore it might not has shown the results.

**FALAA Gold**

FALAA Gold contains Swarna patra in the form of Vark. When patra is added in the formulation again it gets converted in to micro fine powder due to tratuartion. This is evident by SEM results where the particle size ranges from 0.08 $\mu$m – 15 $\mu$m. Swarna is the only metal even after its bhasmikarana remains as Swarna. Only its Particle Size gets reduced. So, The Swarna patra in the Falaa Gold will have similar action as that of Swarna Bhasma.

**Experimental Study**

As mode of action of Rasoushadhi was main area of interest in the study. The Uterotrophic Bioassay an innovative method was adopted to study the mode of action of selected drugs on female albino rat reproductive system. Uterotrophic Bioassay relies on an animal test system for its sensitivity, in which the hypothalamic-pituitary-ovarian axis is not functional, due which low levels of estrogen in the blood. This ensures a low
baseline uterus weight and a greatest range of response to administered oestrogens or chemicals. Immature females after weaning and prior to puberty meet this requirement:

The test substance was administered daily by oral gavage. The animals sacrificed after twenty four hours after the last dose. For estrogen agonists, the mean uterine weight of the treated animal groups relative to the vehicle group was assessed for a statistically significant increase. “A statistically significant increase in the mean uterine weight of a test group has shown a positive response.

The protocol vaginal smear test was included, but as the animals were too small the vagina was observed for opening. In 2-3 animals the vaginal opening was visible during the sacrifice but it was not possible to remove the smear. Hence, the vaginal smear test was not done. The vaginal smear test can be done only if mature rats are used.

**Number of follicles**

Statistical analysis of number of follicles in all 5 groups did not show any significance when compared with control as well as in between groups since value of p was found to be more than 0.05.

**Size of Largest Follicles**

Statistical analysis of size of largest follicles in all 5 groups did not show any significance when compared with control as well as in between groups since value of p was found to be more than 0.05.
**Discussion**

**Thickness of Endometrium**

Statistical analysis of thickness of endometrium in all 5 groups did not show any significance when compared with control as well as in between groups since value of $p$ was found to be more than 0.05.

**Diameter of Uterus**

Statistical analysis of diameter of uterus in all 5 groups did not show any significance when compared with control as well as in between groups since value of $p$ was found to be more than 0.05.

**Follicle Stimulating Hormone Analysis**

As per Uterotrophic Bioassay, FSH and LH measurements are not needed. Though these tests are costly, they were included in the protocol with the interest of learning the effect of selected compounds. Statistical analysis of FSH levels in all 5 groups did not show any significance when compared with control as well as in between groups since value of $p$ was found to be more than 0.05.

**Luteinizing Hormone Analysis**

Statistical analysis of LH levels in all 5 groups did not show any significance when compared with control as well as in between groups since value of $p$ was found to be more than 0.05.
**Discussion**

**Weight of Animals**

Statistical analysis of weight of animals in all treated groups showed significance, when compared with control. Value of p was found to be less than 0.05. But in between treated groups there was no significance. The reason for significant increase in weight may be due to Swarna Bhasma and Kaseesa Bhasma which are well known for their Rasayana effects. The compound formulations also contain Swarna Bhasma and Kaseesa Bhasma.

**Weight of Uterus in all treated groups**

Statistical analysis of weight of uterus in all treated groups showed significance, when compared with control and in between groups. Value of p was found to be less than 0.05. Except FALAA GOLD (Swarna compound formulation) when compared with control. There was no significance found when Kaseesa Bhasma compared with Kaseesa Compound formulation i.e Rajapravartini vati. The reason for non-significance may be Swarna Bhasma content is less in FAALA GOLD (Swarna compound formulation) or other drugs present in the FAALA GOLD were less effective in increasing weight of uterus in selected rat model.

**Though** the statistical analysis of all the groups are not significant with reference to number of follicles, size of largest follicle, thickness of endometrium, diameter of uterus, FSH Levels, LH Levels when compared with control and in between the groups.

Though the results are statistically not significant but there is increase in values of parameters in Swarna Bhasma and Kaseesa Bhasma treated groups. By this we can say that GnRH stimulates (causes) the pituitary gland to produce follicle stimulating hormone.
(FSH), the hormone helps in follicle (egg) development and causing the level of estrogen (the primary female hormone) to rise.

Non uterotrophic effect in animals treated with Falaa Gold, Because in Falaa Gold the quantity of gold is (1mg in 400mg Cap) which is inefficient to exhibit uterotrophic effect.
6. SUMMARY

- In Ayurveda “A single drug may have many applications owing to its diverse actions, just as a man is able to perform various actions”. The present study has tried to evaluate the effects or the mode of action of four Ayurvedic formulations i.e. Swarna Bhasma, Swarna containing compound formulation, Kaseesa Bhasma and Kaseesa containing compound formulations on Reproductive System of the animal model.

- For the study the 3 samples of Swarna Bhasma and Kaseesa Bhasma were purchased from local market. Manufactured by GMP Certified Ayurvedic Companies.

- All Samples of Swarna Bhasma and Kaseesa Bhasma were subjected to Bhasma Pareeksha and further subjected to NPST test.

- The Bhasma which passed all bhasma pareeksha were selected.

- FALAA GOLD (Swarna Bhasma Containing Compound Formulation) and Rajapravartini vati (Kaseesa Bhasma Containing Compound Formulation) were also purchased from local market. Manufactured by GMP Certified Ayurvedic Companies.

- Swarna Bhasma, Kaseesa Bhasma, FALAA GOLD (Swarna Bhasma Containing Compound Formulation) and Rajapravartini vati (Kaseesa Bhasma Containing Compound Formulation) were sent to IIT Mumbai for ICPAES analysis and Kochin University Kerala for XRD, SEM & FTIR analysis.

- The IAEC approval was taken for the study from institutional animal ethics committee.

- The animal study was done following guidelines of Uterotrophic Bioassay.
• Animals were randomly divided into 5 groups containing 6 animals in each group.

• **Group 1**- Received Honey (Control Group), **Group 2**- Received Swarna Bhasma (SB Group) + Honey, **Group 3**- Received FALAA GOLD (Swarna Containing Compound Formulation- Group)+ Honey, **Group 4**- Received Kaseesa Bhasma + Honey (KB Group), **Group 5**- Received Rrajapravartini Vati (Kaseesa Containing Compound Formulation- Group) + Honey.

• The parameters measured are body weight, estimation of FSH and LH hormone levels, uterus weight and Histology of ovaries and uterus.

• The data was analyzed using ANOVA followed by Tukey's Multiple Comparison Test. The values of p was <0.05 were considered significant.

• Based on overall results the Swarna Bhasma and Kaseesa Bhasma have effects on female reproductive system of selected rat model in comparison with their compound formulation as well as with control.
7. CONCLUSION

- Uterine weight shown significant increase in Swarna Bhasma, Kaseesa Bhasma as well as Kaseesa compound formulation treated groups.

- Uterine weight gain is insignificant in the group treated with compound preparations of Swarna (Falaa Gold).

- Significant weight gain of animals seen in all the four groups viz. Swarna Bhasma, FALAA Gold (Swarna Compound Formulation), Kaseesa Bhasma and Rajapravartini vati (Kaseesa Compound Formulations) and the effect is better in comparison with control.

- Effect on size of follicles, number of follicles and endocrine levels (FSH, LH) is insignificant in all the four groups.

- In total it may be concluded that both Swarna Bhasma and Kaseesa Bhasma possess uterotrophic activity.

Recommendations:

- Further studies may be carried out on Swarna Bhasma and Kaseesa bhasma along with suitable Anupana.
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Effect of Swarna Bhasma and Kaseesa Bhasma on Reproductive Function in Female Rats
Photo Plates

Effect of Swarna Bhasma and Kaseesa Bhasma on Reproductive Function in Female Rats

Photo - 6 Kaseesa Bhasma Pareeksha

- Rekha Purna
- Unam
- Nirdhuma
- Varitara
- Niruttha
- Apunarbhaba
Photo 7: NPST of Kaseesa

Clear layer of Kaseesa Bhasma Treated with 5N HNO3

Immediate Spot Patterns of Sample 1, 2, 3 respectively.

Sample 1                  Sample 2                  Sample 3

After 24hrs spot Patterns of Sample 1, 2, 3 respectively.

Sample 1                  Sample 2                  Sample 3
Photo 8: NPST of Swarna Bhasma

Immediate Spot Patterns of Sample 1, 2, 3 respectively.

Sample 1 | Sample 2 | Sample 3
---|---|---
![Image of spot patterns for Sample 1, 2, 3](image1)

After 24hrs spot Pattern sample1, 2, 3 respectively.

Sample 1 | Sample 2 | Sample 3
---|---|---
![Image of spot patterns for Sample 1, 2, 3 after 24hrs](image2)
Photo 9: Swarna Bhasma selected

Photo 10: Kaseesa Bhasma selected

Photo 11: Falaa Gold (Compound formulation of Swarna Bhasma)

Photo 12: Rajapravartini Vati (Compound formulation of Kaseesa Bhasma)
Photo 13: Experimental study

ANIMALS KEPT IN CAGES

Examining Animal for Vaginal Opening

COLLECTION OF BLOOD THROUGH RETRO-ORBITAL PUNCTURE Under ANESTHESIA

DISSECTING ANIMALS
WEIGHING ANIMALS USING DIGITAL WEIGHING MACHINES

WEIGHING OF UTERUS

Storing Blood samples Collected
Marking of animals for proper identification

Drug Administration
Photo 14: Histological study

Histological Study Ovary in Control Group (C)

C1  C2  C3

C4  C5  C6

Photo 15: Histological Study Ovary in Swarna Bhasma Group (S)

S1  S2  S3

S4  S5  S6
Photo -16 Histological Study Ovary in FALAA GOLD (Swarna containing compound formulation) (FG) Group

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<th>FG3</th>
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<table>
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<th>FG4</th>
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Photo 17- Histological study ovaries in Kaseesa Bhasma (KB) group

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<table>
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Effect of Swarna Bhasma and Kaseesa Bhasma on Reproductive Function in Female Rats
Photo 18 Histological Study Ovary in RajapravarthiniVati (Kaseesa containing compound formulation) (R) Group

R1

R2

R3

R4

R5

R6
ANNEXURE I

K.L.E. UNIVERSITY’S
SHRI B.M. KANKANAWADI AYURVED MAHAVIDYALAYA
(A Constituent unit of KLE University, Belgaum)
SHAHAPUR, BELGAUM – 590 003 (KARNATAKA STATE)

Dr. P.A. Patil, Chairman, IAEC
Prof. & HOD, Pharmacology,
J.N.M.C. Belgaum

Mr. Mohammed Sanahulla S.H.
Nominee of CPCSEA

Dr. R.S. Hiremath,
Member Secretary

IAEC Reg. No. 1017/C/06/CPCSEA dated: 19.12.2006

CERTIFICATE

This is to certify that the research project “Effect of Swarna Bhasma and Kaseesa Bhasma on Reproductive function in female rats”

Submitted by Dr. Rajeswari V. Kamat, Ph.D. Scholar has been approved in the Institutional Animal Ethics Committee meeting held on 20th January 2012 resolution No. BMK/IAEC/Res-13/2012 and was permitted to use 30 Female Albino Rats.

You are hereby informed to strictly adhere to the protocol submitted for approval. In case the project needs to be modified later, the modified version of the protocol should be submitted to the committee, stating valid reasons for such modifications for fresh approval.

You are required to keep the account of animals used for the project in specified proforma, Form – D.

You have to submit the brief report to the committee after completion of the project along with Form – D.

Member Secretary
Institutional Animal Ethics Committee
KLE’S Shri B.M. Kankanawadi Ayurved Mahavidyalaya, Shahapur-Belgaum

Ph. No. +91 831-2486286,
Fax : +91 831-2424157
Email : bmkayurveda@rediffmail.com
Web site: www.bmkayurveda.org
ANNEXURE-II

Analytical report of the samples submitted by Dr. Rajeshwari V. Kamat, KLE University, Belgaum, using Inductively Coupled Plasma Atomic Emission Spectroscopy.

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ND MEANS LESS THAN 0.01 PPM

Note: Content of this report is meant for your information only and should not be used for advertisement, evidence or litigation.

To,
Dr. R.S. Hiremath,
Professor,
Dept. Of Rasashastra,
KLE University,
Shahapur,
Belgaum-03
**ANNEXURE III**

**HISTOLOGICAL STUDY REPORT**

---

**JEEVAN REGIONAL DIAGNOSTICS**

REGD. OFFICE: 6331, OPPOSITE LINGARAJ COLLEGE,
COLLEGE ROAD, BELGAUM-02.

---

**Thesis of:** DR. RAJESHWARI V. KAMAT

**Refered by Dr.:** SELF

**Received on:** 02/11/2012

---

**SPECIMEN:** Received eighteen specimens of uterus with ovaries labelled as C1, C2, C3, K1, K2, K3, S1, S2, S3, R1, R2 and R3.

<table>
<thead>
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<th>Diameter of uterus in mm</th>
<th>Thickness of myometrium in mm</th>
<th>Thickness of endometrium in mm</th>
<th>Phase of endometrium</th>
<th>Number of follicles</th>
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**THANKS FOR YOUR REFERENCE**

DR. V.V. VENMI MD,
CONSULTING PATHOLOGIST

---

DR. A.S. AMMANAGI MD DNB MNAMS MSSC,
CONSULTING PATHOLOGIST

---

Contd…
Received eighteen specimens of uterus with ovaries labelled as C4, C5, C6, K4, K5, K6, S4, S5, S6, R4, R5, R6, Ph1, Ph2, Ph3, Ph4, Ph5 and Ph6.

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<th>Thickness of myometrium in mm</th>
<th>Thickness of endometrium in mm</th>
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THANKS FOR YOUR REFERENCE

Dr. V.V. Yenni MD  Consulting Pathologist

Veena

Dr. A.S. Ammanagi MD  DNB  MNAMS  MSSC  Consulting Pathologist

JRD
ANNEXURE IV

The Kits used for FSH & LH Hormone Analysis
Effect of Swarna Bhasma and Kaseesa Bhasma on Reproductive Function in Female Rats

Annexures
ANNEXURE V

DEFINITIONS

**Antioestrogenicity** is the capability of a chemical to suppress the action of estradiol 17β in a mammalian organism.

**Date of birth** is postnatal day 0.

**Dosage** is a general term comprising of dose, its frequency and the duration of dosing.

**Dose** is the amount of test substance administered. For the Uterotrophic Bioassay, the dose is expressed as weight of test substance per unit body weight of test animal per day (e.g. mg/kg body weight/day).

**Maximum Tolerable Dose (MTD)** is the highest amount of a substance that, when introduced into the body does not kill test animals (denoted by DL₅₀) (IUPAC, 1993)

**Oestrogenicity** is the capability of a chemical to act like estradiol 17β in a mammalian organism.

**Postnatal day X** is the Xth day of life after the day of birth.

**Sensitivity** is the proportion of all positive/active substances that are correctly classified by the test. It is a measure of accuracy for a test method that produces categorical results, and is an important consideration in assessing the relevance of a test method.

**Specificity** is the proportion of all negative/inactive substances that are correctly classified by the test. It is a measure of accuracy for a test method that produces categorical results and is an important consideration in assessing the relevance of a test method.

**Uterotrophic** is a term used to describe a positive influence on the growth of uterine tissues.

**Validation** is a scientific process designed to characterize the operational requirements and limitations of a test method and to demonstrate its reliability and relevance for a particular purpose.
ANNEXURE VI

ARTICLE PUBLISHED
Effect of Swarna Bhasma and Kaseesa Bhasma on Reproductive Function in Female Rats
Effect of Swarna Bhasma and Kaseesa Bhasma on Reproductive Function in Female Rats
Effect of Swarna Bhasma on Reproductive (Endocrine) System W.S.R. Immature Female Rat Model

Kamat Rajeshwari V.*, Hiremath R.S.**, Patil P.A.***

Abstract

Swarna, the Sara Lauha is an important, noble metal known to Indians since antiquity. References can be traced back to Charaka and Sushruta Samhita where the noble metal has been used with a wide range of applications. Gold was used before the conception by the couple as geriatric and aphrodisiac, to get a healthy child, after conception at 2nd month to get the desired sex of the child, immediately after birth before the umbilical cord is cut, as a substitute to mothers milk, and during post delivery period for enhancing intelligence, and even at the death bed gold is used. So starting before the conception till the death gold was used in one or the other way. Various formulations of ‘Swarna’ Bhasma are useful as Aphrodisiac, increasing body strength, as rejuvenator and disease alleviators particularly in chronic debilitating diseases like tuberculosis, Bronchial asthma, chronic cough, anemia etc. Human dose for ‘Swarna Bhasma’ is 15 mg. to 30 mg. The present study is an attempt to observe the effect of Swarna bhasma on endocrine system of immature wistar strain female rats with special reference to uterus, ovary, no of follicles FSH & LH & the results are encouraging.

Keywords: Swarna; Swarna bhasma; FSH; LH.

Introduction

Swarna, the Sara Lauha[1] is an important, noble metal known to Indians since antiquity. References can be traced back to Charaka and Sushruta Samhita where the noble metal has been used with a wide range of applications. Gold was used before the conception by the couple as geriatric and aphrodisiac, to get a healthy child, after conception at 2nd month to get the desired sex of the child, immediately after birth before the umbilical cord is cut, as a substitute to mothers milk, and during post delivery period for enhancing intelligence, and even at the death bed gold is used. So starting before the conception till the death gold is used in one or the other way. So gold has a good chemistry with the body. It is said that gold is the Semen of Agni. It is the vigor and virility of Agni. If one consume gold, his vigor and virility will be increased. Ayurveda says kaya means Agni and general medicine means Agni Chikitsa. Gold has important role to play in treatment of many diseases and as a preventive and protective treatment.

Various formulations of ‘Swarna’ (Gold) Bhasma are useful as Aphrodisiac, increasing body strength, as rejuvenator[2] and disease alleviators particularly in chronic debilitating diseases like tuberculosis, Bronchial asthma, chronic cough, anemia[3] etc. Human dose for ‘Swarna Bhasma’ is 15 mg. to 30 mg.[4]

The present study is an attempt to observe the effect of swarna bhasma on endocrine system of immature wistar strain female rats with special reference to uterus, ovary, no of follicles FSH & LH.
Material and Methods

Approval for Animal Study
Approval was taken from institutional Animal ethical committee with approval letter No BMK/IAEC/Res-13/2012.

Animals
12 Wistar strain albino rats of age 16-17 days of birth without evidence of any disease or physical abnormalities & weighing between 20±6gm were procured from licensed breeder KLEU JNMC Animal House Belgaum. The day of the birth being considered as postnatal day 0 & were randomly divided into two groups containing 6 animals in each group. The animals were identified uniquely by marking.

Quarantine
Animals were kept in quarantine for 1 day as per guidelines of Uterotrophic bioassay.

Test Drug
Swarna bhasma was purchased from GMP Certified Ayurvedic Manufacturer with Batch No- P120500101 Date of Manufacture-May 2012. Prepared as per Bharata bhaishajya Ratnakara 5/8357 Ref. Manufacturer Claiming gold % > 95%. For reconfirmation The drug was analyzed for % of gold through ICPAES from IIT Mumbai.

Dose Fixation
Swarna Bhasma maximum therapeutic Human dose is 30 mg. The same dose was converted into animal dose following Paget & Barnes table. Dose was given once a day for 3 consecutive days, at the same time with honey as vehicle through oral gavage.

Control Group
Received vehicle i.e.0.05 ml Honey

Test Group
Received Swarna bhasma 30mg/kg body wt. with 0.05 ml Honey.

Observations
General and clinical observations were made once a day preferably at the same time each day. All animals we observed for mortality, morbidity, general clinical signs such as changes in behavior, fur, eyes, mucus membranes, occurrence of secretions and excretions, lacrimation, pilocrection, pupil size, unusual respiratory.

All animals weighed daily to the nearest 0.1 gm starting prior to the initiation of test drug. The amount of food & water consumed daily are expressed in grams per rat/day and ml per rat/day respectively.

Collection of Blood for FSH & LH Assay
24 hrs after the last dose through retro orbital puncture under anesthesia blood was collected and sent for hormonal analysis.

Dissection
24 hrs after the last dose rats were humanely sacrificed.

Uterus Weight
Each uterus is transferred to a uniquely marked and weighed container. Then the uterus were transferred to a uniquely marked formalin container & sent for histological study.

Ovaries
Each pair of ovaries collected were transferred to a uniquely marked formalin container & sent for histological counting of follicles.
Table 1: Showing Statistical Results No of Follicles

<table>
<thead>
<tr>
<th>Significance</th>
<th>Swarna Bhasma</th>
<th>Control</th>
<th>Particulars</th>
</tr>
</thead>
<tbody>
<tr>
<td>55.50</td>
<td>29.00</td>
<td>Mean</td>
<td></td>
</tr>
<tr>
<td>23.96</td>
<td>7.823</td>
<td>Std. Deviation</td>
<td></td>
</tr>
<tr>
<td>Ns</td>
<td>0.0800</td>
<td>P value</td>
<td></td>
</tr>
</tbody>
</table>

Graph 1: Showing No of Follicles

Table 2: Showing Statistical Result of Largest Follicle Size

<table>
<thead>
<tr>
<th>Significance</th>
<th>Swarna Bhasma</th>
<th>Control</th>
<th>Particulars</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2833</td>
<td>0.2917</td>
<td>Mean</td>
<td></td>
</tr>
<tr>
<td>0.07528</td>
<td>0.05845</td>
<td>Std. Deviation</td>
<td></td>
</tr>
<tr>
<td>Ns</td>
<td>0.9816</td>
<td>P value</td>
<td></td>
</tr>
</tbody>
</table>

Graph 2: Showing Result of Largest Follicle Size

Table 3: Showing Statistical Result Thickness of Endometrium

<table>
<thead>
<tr>
<th>Significance</th>
<th>Swarna Bhasma</th>
<th>Control</th>
<th>Particulars</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.6500</td>
<td>0.4333</td>
<td>Mean</td>
<td></td>
</tr>
<tr>
<td>0.1643</td>
<td>0.1211</td>
<td>Std. Deviation</td>
<td></td>
</tr>
<tr>
<td>Ns</td>
<td>0.1293</td>
<td>P value</td>
<td></td>
</tr>
</tbody>
</table>
Annexures

Graph 3: Showing Thickness of Endometrium

Table 4: Showing Statistical Result Diameter of Uterus in mm

<table>
<thead>
<tr>
<th>Significance</th>
<th>Swarna Bhasma</th>
<th>Control</th>
<th>Particulars</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.867</td>
<td>4.017</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>0.2503</td>
<td>0.7731</td>
<td>Std. Deviation</td>
</tr>
<tr>
<td>Ns</td>
<td>0.6611</td>
<td>P value</td>
<td></td>
</tr>
</tbody>
</table>

Graph 4: Showing Diameter of Uterus in mm

Table 5: Showing Statistical Result FSH

<table>
<thead>
<tr>
<th>Significance</th>
<th>Swarna Bhasma</th>
<th>Control</th>
<th>Particulars</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.6433</td>
<td>0.6667</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>0.4500</td>
<td>0.1506</td>
<td>Std. Deviation</td>
</tr>
<tr>
<td>Ns</td>
<td>0.4564</td>
<td>P value</td>
<td></td>
</tr>
</tbody>
</table>
Annexures

Graph 5: Showing Result FSH (Follicle Stimulating Hormone)

Table 6: Showing Statistical Result LH (Luteinizing Hormone)

<table>
<thead>
<tr>
<th>Significance</th>
<th>Swarna Bhasma</th>
<th>Control</th>
<th>Particulars</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.160</td>
<td>0.9167</td>
<td>Mean</td>
<td></td>
</tr>
<tr>
<td>0.9652</td>
<td>0.6113</td>
<td>Std. Deviation</td>
<td></td>
</tr>
<tr>
<td>Ns</td>
<td>0.8935</td>
<td>P value</td>
<td></td>
</tr>
</tbody>
</table>

Graph 6: Showing Result LH (Luteinizing Hormone)

Discussion

It is essential to prove mode of action of ayurvedic drugs scientifically by modern parameters, through which Ayurveda can excel in the current era as evidence based & well documented system of medicine. The reason behind selecting immature rat model is the Uterotrophic Bioassay relies for its sensitivity on an animal test system in which the hypothalamic-pituitary-ovarian axis is not functional. (Test No. 440: Uterotrophic Bioassay in Rodents A short-term screening test for oestrogenic properties)

Follicle-stimulating hormone (FSH) is a hormone found in humans and other animals. It is synthesized and secreted by gonadotrophs of the anterior pituitary gland. FSH regulates the development, growth, pubertal maturation and reproductive processes of the body. FSH and luteinizing hormone (LH) act synergistically in reproduction. In the present study low FSH levels indicate early maturation of the follicle.
in the test group.

Luteinizing hormone (LH, also known as lutropin[7] and sometimes lutrophin[8]) is a hormone produced by gonadotroph cells in the anterior pituitary gland. In females, an acute rise of LH ("LH surge") triggers ovulation[9] and development of the corpus luteum. In males, where LH had also been called interstitial cell-stimulating hormone (ICSH).[10] It acts synergistically with FSH. FSH & LH levels are normally low during childhood. During the reproductive years, typical levels are between 1-20 IU/L. In the present study LH levels are increased in the Swarna bhasma group which indicates the early ovulation, development of corpus luteum & increase in the thickness of the uterus.

Statistical Analysis

One-way ANOVA followed by post hoc multiple comparisons (Dunnet's) test.

Conclusion

No of follicles, thickness of uterus & LH levels though statistically not significant but graphical representation shows remarkable increase in Swarna bhasma group in comparison to control. Size of the largest follicle, Diameter of the uterus FSH levels were more in the control group in comparison to test group. These results conclude that Swarna bhasma is having its action on female reproductive endocrine system in immature Wistar strain female rat.

References

6. Follicle-Stimulating Hormone.
7. Lutropin at eMedicine Dictionary
9. Jump up to a b Physiology at MCG 5/5ch9/5ch9_5
Effect of Swarna Bhasma and Kaseesa Bhasma on Reproductive Function in Female Rats
Effect of Swarna Bhasma and Kaseesa Bhasma on Reproductive Function in Female Rats
From desk of Editors..................

With great pleasure, we want to place before you third issue (July - September) of International Journal of Pharmaceutical Research (ISSN-0975-2366) for the year 2014. The excellent response provided by various institutes, colleges and industrial organization has given us enough motivation and encouragement to put before you another set of IJPR issue at your service. By now we have accrued enough experience and have understood to a larger extent the need of institutes, colleges & industry. IJPR is actively involved in publishing new review articles & research articles since last three years and has made special name at national & international level. IJPR is indexed in Chemical Abstract Service (CAS), Scopus, Embase, Ulrich International Periodical Dictionary & Indian Science Abstract (ISA).

The breaking news is that IJPR is has secured 5 – years impact factor 0.973

IJPR is now available as fully dynamic website starting from online research paper submission to final publication of research article, which is aimed to provide a platform for peer-reviewed high quality manuscripts and rapid processing of these manuscripts toward publication. IJPR provides a common platform for all professionals to facilitate interaction, to update our knowledge and to March into the service of mankind. The Advisory & Editorial team constituted by members from the entire specialization of pharmaceutical science are working hard to update IJPR with new technology. We strongly believe that this cooperation and unity will help in providing a high quality platform for the IJPR. It would be our pleasure to thank all the members of the Advisory team, editorial team, reviewers, authors for their cooperation and support extended in bringing this issue of the journal possible. At the end, we hope that this issue of IJPR would fortify the bond between industry, researcher & academia fostering evolution of the pharmaceutical world.

With Season’s greetings........

Editor in chief

International Journal of Pharmaceutical Research
Effect of Swarna Bhasma and Kaseesa Bhasma on Reproductive Function in Female Rats

Research Article

Evaluation of Market Samples of ‘Kaseesa Bhasma’ Using ‘Namburi Phased Spot Test’

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²Dept of Pharmacology University of Sains Malaysia KLE International Medical Programme School Belgaum, Karnataka

ABSTRACT

Kaseesa bhasma (ferrous sulphate), an unique herbo-mineral preparation used traditionally in iron deficiency anemia, vitiligo, dysuria, renal calculi, worm infestation, anti-pyretic, spleenomegaly and in amenorrhea. Many pharmaceutical companies manufacture Kaseesa bhasma but whether the quality of all remains same or not is always doubtful. This doubt can be cleared by using classical bhasma parikshas (Tests to assess Bhasma) like Rekhaparnata (Passing through the furrows of the fingers), Vaaritara (floating on water),Unama (Rice grain kept on floating bhasma ) and modern analytical techniques like NPST. Thus, Kaseesa bhasma prepared by classical reference in our department along with 2 market samples were subjected to above tests and the results were compared. There was considerable difference in the bhasma parikshas and NPST spot pattern among all the 3 samples. The bhasma prepared in our department had nearest results to standard NPST of Kaseesa bhasma.

Keywords: Kaseesa bhasma, Bhasma parikshas, Namburi phased spot test (NPST), ferrous Sulphate

INTRODUCTION

Kaseesa bhasma (ferrous sulphate), an unique herbo-mineral preparation used traditionally in iron deficiency anemia, vitiligo, dysuria, renal calculi, worm infestation, anti-pyretic, spleenomegaly and in amenorrhea.¹,²

The Namburi Phased Spot Test (NPST), a spot test based on a chemical reaction, is a new technique for assessing the quality of a prepared bhasma. When a drop of clear solution of a substance under examination (Bhasma or Sindhura) is put on specially prepared chemical reacting papers, a spot appears which manifests a series of colour and pattern changes. In chemistry, techniques involving spot tests or chromatography are widely used. The NPST involves observations of the spot and its colour, at three successive phases spread over three different time intervals. It thus has the advantage of measuring sensitivity of reactions at different time intervals. In other words, it constitutes a method to study or detect, every second or even fraction of a second, continual chemical reactions taking place gradually between two chemical substances on static media. The technique was developed and standardized by Dr. Namburi Hanumantha Rao in 1970, it has been accepted by CCRAS, New Delhi.

NPST and other classical tests were performed on samples of Kaseesa Bhasma. The first prepared classically and two other market samples, also said to be prepared by same reference in order to compare and evaluate their quality.

MATERIALS AND METHODS

A three-part methodology was used:

- Obtaining samples of 3 samples Kaseesa bhasma: first prepared classically, two others purchased from market.
- Subjecting all samples to classical bhasma parikshas.
- Subjecting all samples to NPST.

Preparation of Kaseesa (Ferrous Sulphate) bhasma

Authenticated Kaseesa was taken from the department of Rasashastra, K. L. E. U. Shri B. M. K. Ayurveda Mahavidyalaya, Shahapur, Belgaum, Karnataka, Kasisa She...
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Annexures

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dharu (purification of kasisa) was carried out by 3 hour soaking with Bhringaraja Swarasas (extract of Eclipta Alba Niss). For preparation of Kaseesa bhasma purified Kaseesa was triturated with lemon juice and subjected to heat treatment. The procedure is repeated till the prepared bhasma passes all classical bhasma pareekha.

Bhasma parikshas

The prepared Kaseesa bhasma (sample number 1), and the three market samples (numbers 2 and 3) were subjected to various classical bhasma parikshas like Rekhapurnata, Varitara, Unama, Nirdhuma and Niswadu (Table1)

Table 1: Analysis of 3 samples of Kaseesa Bhasma

<table>
<thead>
<tr>
<th>Parameter / Test</th>
<th>Sample 1(D)</th>
<th>Sample 2(B)</th>
<th>Sample 3(V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>Dark brown</td>
<td>Slightly light brown</td>
<td>Reddish</td>
</tr>
<tr>
<td>Touch</td>
<td>Smooth</td>
<td>Smooth</td>
<td>Fine</td>
</tr>
<tr>
<td>Taste</td>
<td>Absent</td>
<td>Slight sour taste</td>
<td>Absent</td>
</tr>
<tr>
<td>Odour</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Rekhapurnatwa</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Varitara</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Unama</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Nirdhuma</td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
</tr>
</tbody>
</table>

Namburi Phased Spot Test

All the 3 samples were subjected to NPST. Initially, 0.25 gm of each bhasma sample was placed in a test tube and heated till bottom appears red before treating with reagents. Then samples were cooled then 0.5 ml of 5N HNO3 was added to it drop by drop again heated for one minute. It was kept in a stand for 72 hours, during which time it was shaken occasionally. It was then allowed to settle while a clear layer formed (Figure 1). One drop was taken from the clear layer and placed on 10% potassium iodide paper (prepared using Whatman’s filter paper no.1), colour changes in the paper was observed over 3 time periods ref. (Figure 2)

Figure 1: Clear layer of Kaseesa Bhasma Treated with 5N HNO3

Figure 2: Immediate spot Pattern sample 1, 2, 3 respectively.

Figure 3: After 1 day spot Pattern sample 1, 2, 3 Respectively.
Table 2: NPST of 3 samples of Kaseesa Bhasma

<table>
<thead>
<tr>
<th>Observation</th>
<th>Sample 1 (D)</th>
<th>Sample 2 (B)</th>
<th>Sample 3 (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPST Observations:</td>
<td>Wet periphery forms followed by a thick blue circle in the centre of the spot. But wet spot was not much wide.</td>
<td>Wet periphery appears followed by light blue stain in the centre.</td>
<td>Wet periphery appears followed by light blue circle in the centre.</td>
</tr>
<tr>
<td>I Phase (0-5 min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II Phase (5-20 min)</td>
<td>Wet periphery faded with reduction in the brightness of blue circle.</td>
<td>Wet periphery became light blue at the end of II phase.</td>
<td>Wet periphery faded away in this phase.</td>
</tr>
<tr>
<td>III Phase (20 min-1 day)</td>
<td>Dark Blue central circle with light blue periphery.</td>
<td>Light blue centre with light brown periphery.</td>
<td>Dark blue center with light blue periphery.</td>
</tr>
</tbody>
</table>

Table 3: Standard NPST of Kaseesa bhasma

<table>
<thead>
<tr>
<th>Phase I (0-5 min)</th>
<th>Phase II (5-20min)</th>
<th>Phase III (20 min-1 day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet periphery followed by a thick blue circle in the centre of the spot.</td>
<td>Wet periphery blue circle</td>
<td>Dark Blue central circle with light blue periphery.</td>
</tr>
</tbody>
</table>

Observations of NPST

There was lot of difference in the spot pattern of all the 3 samples when compared to standard NPST of Kaseesa bhasma (Table 2).

DISCUSSION

The colours of the all 3 Samples differ a great varying from dark brown to Reddish. The wide range of colour difference may be due to the drug used for purification and also on the number of puta given. The touch of the bhasma showed that samples 1 and 2 are much smoother than 3rd sample. In sample 2 sour taste was present which may indicate improper formation of the bhasma. This was substantiated when it evoked fumes in the Nirdhuma test. Though all samples passed the Varitara test, Unama test.

In NPST the desired results were seen in all 3 samples, but sample 1 showed more accurate results compared to the others [figure 2&3]. The results seemed to be similar, but were not the same - an advantage of conducting NPST over other classical bhashmaparikshas. The classical tests cannot differentiate between bhasmas chemically, but in NPST, as the test is chemical reaction-based, with specific results for specific bhasmas, we can differentiate between bhasmas clearly. This technique is very helpful for quality assessment of Bhasma as per the standards of Rasashastra. In other words, bhasmas can be identified by their name given in Rasashastra by virtue of their quality differences, but not chemically. It is such a simple test that it can be carried out with minimum set up and requirements. CCRAS has also accepted the monograph of NPST, and so the quality of Bhasma can be checked before being used therapeutically. In the present study, though the bhasma was said to be prepared by same method, there was lot of difference in bhasma colour, and according to NPST only sample 1, prepared in our department, gave results in accordance with the text.

CONCLUSION

NPST is a chemical reaction based test helpful for quality assessment of bhasma. In all these 3 samples, sample 1 showed results nearer compared to standard NPST, which indicates the genuinity of the sample.

REFERENCES

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