Pharmaceutico-Analytical Study of Muktashukti Pishti and Muktashukti bhasma and Comparative Evaluation of their Relative Oral Bioavailability

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Authors' contributions

This work was carried out in collaboration between all authors. Author SK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author AW managed the analyses of the study. Authors BR and DR managed the literature searches. All authors read and approved the final manuscript.

ABSTRACT

Background: Shukti (Oyster) is a very commonly occurring calcium form. It is rich source of calcium & minerals. As per text it can be converted into two forms which are bhasma (calcinated ash) and pishti (powdered form without agni). These forms may have different rate of absorption. This needs to be studied.

Aim: To study Pharmaceutico-analytical study of Muktashukti pishti & Muktashukti bhasma and comparative evaluation of their relative oral bioavailability.

Materials and methods: The two formulations will be prepared from shukti (oyster). By triturating with Gulabjala Muktashukti pishti will be prepared and by traditional puta method Muktashukti bhasma will be prepared. The prepared formulations will be assessed for Bhasma Parksha mentioned in Ayurveda. Organolectic characters, physicochemical parameters and Particle size distribution analysis, SEM-EDX (Scanning Electron Microscopy, Energy Dispersive X-Ray Analysis), FTIR (Fourier-transform infrared spectroscopy), XRD (X-Ray Diffraction), GCMS (Gas
Chromatography Mass Spectrometry) will be evaluated. To assess the relative oral bioavailability of Mukta shukti & Mukta bhasma study will be conducted in healthy volunteers and will be compared with the standard calcium supplement. The study will be conducted in between two test groups and standard group.

**Observation and results:** The analytical parameters will be assessed and compared in Mukta bhasma and Mukta shukti. For relative oral bioavailability Blood serum calcium will be assessed in all three groups. By applying unpaired “t” Test, One-way ANOVA the statistical significance can be measured.

**Conclusion:** The pharmaceutical & analytical study of Mukta shukti and Mukta bhasma will provide the standard parameters and clinical comparative evaluation with standard will generate evidence for better bioavailability.

**Keywords:** Mukta shukti; Mukta bhasma, analysis; bioavailability.

1. **INTRODUCTION**

Rasashastra & Bhaishajya Kalpana is one among the branches of Ayurveda, which deals with Ayurvedic pharmaceutics. Rasashastra deals with pharmaceutical preparation of Ayurveda related to metallic origin [1]. Most emphasis is given with respect to the therapeutic uses of mercurial, mineral and metallic medicines including calcium containing formulations specified for various disease conditions [2].

Bhasma is a metallic or mineral preparation treated with specific liquid which are mostly juice, decoction or urine of animals & then exposed to quantum of heat according to their suitable properties known as puta. It is an ash obtained through incineration. The raw material undergoes an elaborate process of purification (shodhan) followed by maran. The end product i.e. bhasma is expected to be a non-toxic material which can be readily absorbed & assimilated.

**Pishti** is a fine powder of medicine that absorbs in body easily and possess similar efficacy like that of bhasma. The same purified drug can be used for making pishti as used for making bhasma but there is difference in preparation method and their potency. Pishti also has quick absorption and assimilation because of micro-fine particles like bhasma [3]. Use of metallic & mineral preparations for maintaining health & curing diseases is a unique feature of rasa shishra. Sudhavarga dravya are grouping of drugs that possess high calcium content. It includes Shankha, Shukti, Pravala, Godanti, Dugdhaspahan, Samudraphena, and Mrudgarshrunga [4].

Calcium is a trace element that every living organism need. It is the most essential nutrient in the human body [5]. Human needs calcium to building & maintaining strong bones & 99% of the body calcium is present in the bones & teeth. It is also useful for maintaining healthy communication between the brain & body parts. It has very essential role in physiological function of regulation of gastro intestinal secretions, muscular movement, bone structure and cardiac physiology [6].

Shukti is a readily available & most cost-effective drug from sudhavarga. Mukta shukti and jalashukti are the two types of shukti. Mukta shukti is the outer hard covering shell of mukta. This provides mukta protection, nutrition and structural frame for its survival and hence called by synonyms muktagritha, muktamata and muktamandira. The shukti which not contain mukta or Mollusa into it and which is obtained from sea is called as jalashukti. Shukti is an source of various elements like zinc, iron, calcium, selenium as well as vitamin A and vitamin B12; dietary supplements may contain calcium carbonate from it [7]. “Shuktija yoga” is mention in visarpachikitsa externally for pradeha [8]. It is used in netraroga for anjana karma [9]. Shukti in many formulations cures diseases like shoola, amlapitta, grahani etc [10]. Ayurved prakash explain shukti in the preparation of “kshara bandha” [11]. Mukta shukti bhasma is having cooling effect. It is useful in Heart disease and giving strength to brain it is useful in pittaj vyadhi, fever & flatulence [12]. Mukta shukti pishti reduce excess pitta and heat due to its sheeta virya. It is beneficial in heart burn, abdominal pain, anorexia, calcium deficiency etc.

The analytical study & the therapeutic efficacy of the drug is already mentioned and established with research studies but, the Mukta shukti pishti & Mukta bhasma may differ in the analytical parameters. However, same material undergoes different pharmaceutical methods to obtain different end product, may shows
difference in bioavailability & thus therapeutic efficacy also. Considering this the study has been planned to assess the relative oral bioavailability of Muktashukti pishti & Muktashukti bhasma along with standard calcium supplement.

For all life stages Calcium is very essential compound. Sudhavargadravya possess high calcium content. Out of which Shukti is easily available & cheap source. Pharmaceutico-analytical study of the Muktashukti pishti & Muktashukti bhasma was performed in previous works but the bioavailability study of these both formulations was not done. However, their therapeutic efficacy may vary as per method of preparation. Considering this, the study is planned with development of standard operating process and for their relative oral bioavailability with standards. The drug given through oral route appears in some quantity only, in the blood plasma [13]. In this study the plasma concentration will be assessed in all three groups. Out of these herbo-mineral calcium supplements, one which shows significant bioavailability with that of standard calcium supplement, can be used safely without giving any side effects as standard calcium supplement shows side effects like constipation & abdominal discomfort.

2. MATERIALS AND METHODS

Study design: Randomized single blind controlled study

Sample size: The sample size calculation for a bioavailability and bioequivalent study is dependent on multiple factors like power, intra subject coefficient of variation, expected geometric mean ratio.

According to C. Bhupati and V.H. Vajha. (STATISTICA, anno LXXVII, n.1, 2017), power of 85% would be reasonable for bioavailability study to conduct on healthy volunteers. By considering the values of Lower Bound (LL) =0.80, Upper bound (UL) = 1.25, Alpha=0.05, Geo Mean Ratio (GMR) = 0.947, Coefficient of Variation (CV) = 0.239 as fixed, the sample size can be calculated as below.

Pharmaceutical study: pharmaceutical preparation of Muktashukti bhasma & Muktashukti pishti will be prepared. It will be done by following steps.

I) Procurement and Authentication of Raw materials:
1. Shukti will be procured from Shri Shaila Agency, Nagpur and will be authenticated by the Department of Rasashashtra (MGACH & RC).
2. Kumari & Gulabpushpa will be collected from medicinal plants garden (MGACH & RC), and primarily authenticated by Dravyaguna Department.
3. Kanji & Gulabjal will be prepared in Dattatraya Rasashala which is required for Shodhan of Mukta shukti & preparation of Muktashukti pishti respectively.

II) Shodhana (purification) of Shukti: [14]
Small pieces of shukti will be made with the help of mortar & pestle
These pieces will tied in a clean cloth to make a pottali
The potalli will be subjected to swedan in vessel containing kanji for 3 hrs (1 yam)
After it shukti pieces will be washed with warm water & dried.

III) Preparation of Muktashukti pishti [15]
Shodhita muktashukti will be pounded in khalva yantra
Triturating will be done in khalva yantra till 21 days by adding Gulabjal into it.

IV) Marana (incineration) of Shukti: [16]
Shodhit shukti pieces will be crushed again in a khalvayantra
Kumari swarasa will be added into it to make a paste
Chakrika will be made from it & allowing to dry
Prepared chakrika will be kept in sarava
Sandhi lepan will be done and allowing to dry the sarava. Then it will be subjected to heating for giving one gajaputa till sidhi pariksha attains.

**Analytical study:** For analytical study organoleptic characters and physicochemical parameters and other sophisticated tests like Particle size distribution analysis, SEM –EDX, FTIR, XRD, and GCMS will be done [17].

**Study Parameters [18]**

**Analytical study:** Under analytical study the organoleptic study will be performed under following heads by using the sense organs

- **Specifications** –
  - a. Colour
  - b. Odour
  - c. Taste
  - d. Touch

- **Physico-Chemical analysis**
  1. pH(10% aqueous extract)
  2. Loss on drying at 105°C
  3. Ash value analysis under this , Total ash value , Water soluble ash and Acid-insoluble ash will be analysed
  4. Water soluble extractive values and alcohol soluble extractive values will be calculated

**Sophisticated Instrumental analysis**

1. Particle size distribution analysis
2. SEM –EDX
3. FTIR
4. XRD
5. GCMS

**Bioavailability study:** It will be randomized single blinded study in which 30 healthy Volunteers in each group will be selected (total 90 volunteers) from Swastharakshan OPD, Mahatma Gandhi Ayurvedic College Hospital and Research Centre, salod (H), Wardha.

**Eligibility criteria:** Age group from 20 to 40 years of volunteers will be taken in the study. The screening parameters for this will be physical examination and complete blood count (CBC), blood sugar, liver function test, Kidney function test, lipid profile, blood pressure. The volunteers with normal values will be selected for the study.

**Interventions:** In total 90 volunteers one group with 30 volunteers will be standard group in which standard Calcium supplement were given 500mg once a day before meal, second group with 30 volunteers Muktashukti pishhti will be given 500mg once a day before meal and the third group with 30 volunteers Muktashukti bhasma will be given 500mg once a day before meal. The study will be conducted for 15 days.

**Investigation during treatment:** Complete blood count, Liver Function test, Kidney Function test, Lipid profile, Blood sugar, Urine routine and microscopic, Blood Serum Calcium level will be done for screening.

**Criteria for discontinuing or modifying allocated interventions:** If patient having any problem related to consumption of medicine or having any sensitivity will be withdraw from study.

**Follow up period after treatment:** After 24 hours, 3rd day, 7th day, 15th day of drug administration.

**Implementation:** Principal invigilator will allocate and enroll the patient.

**Observation & Results:** The pharmaceutically prepared Muktashukti pishhti and Muktashukti bhasma will be analyzed for organoleptic parameters and physicochemical parameters. The parameters will be compared. The sophisticated instrumental analysis, SEM –EDX, FTIR, XRD and GCMS of Muktashukti pishhti and Muktashukti bhasma will be done and compared as per the results obtained.

**Table 1. The sample size and power**

| Sample | 54 | 50 | 47 | 44 | 35 | 30 | 26 | 24 |
|--------|----|----|----|----|----|----|----|----|
| Power  | 97.9 | 97.0 | 96.0 | 95.0 | 90.0 | 85.0 | 80.0 | 76.6 |
The relative oral bioavailability of *Muktashuki pishti* and *Muktashuki bhasma* in comparison with standard calcium will be observed.

**Statistical analysis:** Statistical analysis will be done by applying unpaired *t* Test & One-way ANOVA. Unpaired *t* test will be applied for pre and post assessment of Blood serum calcium. One way ANOVA will be applied for assessment of statistical significance related to Blood serum calcium, in between three groups.

### Table 2. Dose and frequency

| Sr no | Group            | Sample size | Intervention                | Dose & frequency | Anupan (Vehicle) | Duration |
|-------|------------------|-------------|-----------------------------|------------------|------------------|----------|
| 1.    | Standard group   | 30 volunteers | Standard calcium compound (SDC) | 500mg (OD) before meal | Water            | 15 days  |
| 2.    | Test group 1     | 30 volunteers | *Muktashuki pishti* (MSP)    | 500mg (OD) before meal | Water            | 15 days  |
| 3.    | Test group 2     | 30 volunteers | *Muktashuki bhasma* (MSB)    | 500mg (OD) before meal | Water            | 15 days  |

### Table 3. Blood collection after administration of drug

| Group | Blood collection after administration of drug |
|-------|-----------------------------------------------|
| SDC   | 00 24 hrs 3rd day 7th day 15th day            |
| MSP   | 00 24 hrs 3rd day 7th day 15th day            |
| MSB   | 00 24 hrs 3rd day 7th day 15th day            |

### Table 4. Coding of blood sample of Group SDC

| Group SDC (n=30) | Blood collection after administration of drug |
|------------------|-----------------------------------------------|
| SDC1             | SDC1-00 SDC1-24 SDC1-3 SDC1-7 SDC1-15        |
| SDC2             | SDC2-00 SDC2-24 SDC2-3 SDC2-7 SDC2-15        |
| SDC3             | SDC3-00 SDC3-24 SDC3-3 SDC3-7 SDC3-15        |
| SDC4             | SDC4-00 SDC4-24 SDC4-3 SDC4-7 SDC4-15        |
| SDC5             | SDC5-00 SDC5-24 SDC5-3 SDC5-7 SDC5-15        |
| SDC6             | SDC6-00 SDC6-24 SDC6-3 SDC6-7 SDC6-15        |
| SDC7             | SDC7-00 SDC7-24 SDC7-3 SDC7-7 SDC7-15        |
| SDC8             | SDC8-00 SDC8-24 SDC8-3 SDC8-7 SDC8-15        |
| SDC9             | SDC9-00 SDC9-24 SDC9-3 SDC9-7 SDC9-15        |
| SDC10            | SDC10-00 SDC10-24 SDC10-3 SDC10-7 SDC10-15   |
| SDC11            | SDC11-00 SDC11-24 SDC11-3 SDC11-7 SDC11-15   |
| SDC12            | SDC12-00 SDC12-24 SDC12-3 SDC12-7 SDC12-15   |
| SDC13            | SDC13-00 SDC13-24 SDC13-3 SDC13-7 SDC13-15   |
| SDC14            | SDC14-00 SDC14-24 SDC14-3 SDC14-7 SDC14-15   |
| SDC15            | SDC15-00 SDC15-24 SDC15-3 SDC15-7 SDC15-15   |
| SDC16            | SDC16-00 SDC16-24 SDC16-3 SDC16-7 SDC16-15   |
| SDC17            | SDC17-00 SDC17-24 SDC17-3 SDC17-7 SDC17-15   |
| SDC18            | SDC18-00 SDC18-24 SDC18-3 SDC18-7 SDC18-15   |
| SDC19            | SDC19-00 SDC19-24 SDC19-3 SDC19-7 SDC19-15   |
| SDC20            | SDC20-00 SDC20-24 SDC20-3 SDC20-7 SDC20-15   |
| SDC21            | SDC21-00 SDC21-24 SDC21-3 SDC21-7 SDC21-15   |
| SDC22            | SDC22-00 SDC22-24 SDC22-3 SDC22-7 SDC22-15   |
| SDC23            | SDC23-00 SDC23-24 SDC23-3 SDC23-7 SDC23-15   |
| SDC24            | SDC24-00 SDC24-24 SDC24-3 SDC24-7 SDC24-15   |
| SDC25            | SDC25-00 SDC25-24 SDC25-3 SDC25-7 SDC25-15   |
| SDC26            | SDC26-00 SDC26-24 SDC26-3 SDC26-7 SDC26-15   |
| SDC27            | SDC27-00 SDC27-24 SDC27-3 SDC27-7 SDC27-15   |
| SDC28            | SDC28-00 SDC28-24 SDC28-3 SDC28-7 SDC28-15   |
| SDC29            | SDC29-00 SDC29-24 SDC29-3 SDC29-7 SDC29-15   |
| SDC30            | SDC30-00 SDC30-24 SDC30-3 SDC30-7 SDC30-15   |
3. DISCUSSION

Ayurveda formulations are becoming popular throughout the world. Rising population, cost effectiveness, less side effects, available at all places are few remarkable causes regarding the use of herbal and mineral drugs as a source of medicines and health supplements [19]. With growing importance, its safety and efficacy studies must be conducted for global acceptance [20]. By incineration the bioavailability may be increased and the drug action may be potentiated. [21] The analysis of MSB and MSP will be compared. In both the samples organoleptic characters that is color, odor, taste will be assessed. Particle size will be assessed, which is a major parameter by means of which rate of absorption can be assessed in MSB and MSP. From scanning Electron Microscopy Energy Dispersive X-Ray Analyzer (SEM EDX) is elemental identification along with quantitative composition can be finding out in MSB and MSP [22]. By Fourier Transform Infrared Spectroscopy (FTIR) chemical bonds will be identified in MSP and MSB [23]. With the help of X-Ray Diffraction (XRD) the crystalline structures of the molecule will be recognized in both the samples that are MSP and MSB [24]. GC-MS technique will be used to analyze complex organic and biochemical mixtures between MSP and MSB [25]. Related studies of standardization of few ayurvedic drugs were reported [26,27]. Pharmaceutico-analytical studies and reviews by Khatib et. al. were reviewed [28,29]. The relative oral bioavailability between MSP & MSB and standard calcium will be assessed. The Herbo mineral formulations are the most efficacious formulations [30]. However, the assessment will be done by evaluation of serum calcium in all of the three groups. The plasma concentration of the serum calcium will be plotted against time in all the three groups. It is represented by the curve, known as area under curve [31].

| Group MSP(n=30) | Blood collection after administration of drug |
|----------------|---------------------------------------------|
|                | 00  | 24hrs | 3rd day | 7th day | 15th day |
| MSP1           | MSP1-00 | MSP1-24 | MSP1-3  | MSP1-7  | MSP1-15  |
| MSP2           | MSP2-00 | MSP2-24 | MSP2-3  | MSP2-7  | MSP2-15  |
| MSP3           | MSP3-00 | MSP3-24 | MSP3-3  | MSP3-7  | MSP3-15  |
| MSP4           | MSP4-00 | MSP4-24 | MSP4-3  | MSP4-7  | MSP4-15  |
| MSP5           | MSP5-00 | MSP5-24 | MSP5-3  | MSP5-7  | MSP5-15  |
| MSP6           | MSP6-00 | MSP6-24 | MSP6-3  | MSP6-7  | MSP6-15  |
| MSP7           | MSP7-00 | MSP7-24 | MSP7-3  | MSP7-7  | MSP7-15  |
| MSP8           | MSP8-00 | MSP8-24 | MSP8-3  | MSP8-7  | MSP8-15  |
| MSP9           | MSP9-00 | MSP9-24 | MSP9-3  | MSP9-7  | MSP9-15  |
| MSP10          | MSP10-00 | MSP10-24 | MSP10-3 | MSP10-7 | MSP10-15 |
| MSP11          | MSP11-00 | MSP11-24 | MSP11-3 | MSP11-7 | MSP11-15 |
| MSP12          | MSP12-00 | MSP12-24 | MSP12-3 | MSP12-7 | MSP12-15 |
| MSP13          | MSP13-00 | MSP13-24 | MSP13-3 | MSP13-7 | MSP13-15 |
| MSP14          | MSP14-00 | MSP14-24 | MSP14-3 | MSP14-7 | MSP14-15 |
| MSP15          | MSP15-00 | MSP15-24 | MSP15-3 | MSP15-7 | MSP15-15 |
| MSP16          | MSP16-00 | MSP16-24 | MSP16-3 | MSP16-7 | MSP16-15 |
| MSP17          | MSP17-00 | MSP17-24 | MSP17-3 | MSP17-7 | MSP17-15 |
| MSP18          | MSP18-00 | MSP18-24 | MSP18-3 | MSP18-7 | MSP18-15 |
| MSP19          | MSP19-00 | MSP19-24 | MSP19-3 | MSP19-7 | MSP19-15 |
| MSP20          | MSP20-00 | MSP20-24 | MSP20-3 | MSP20-7 | MSP20-15 |
| MSP21          | MSP21-00 | MSP21-24 | MSP21-3 | MSP21-7 | MSP21-15 |
| MSP22          | MSP22-00 | MSP22-24 | MSP22-3 | MSP22-7 | MSP22-15 |
| MSP23          | MSP23-00 | MSP23-24 | MSP23-3 | MSP23-7 | MSP23-15 |
| MSP24          | MSP24-00 | MSP24-24 | MSP24-3 | MSP24-7 | MSP24-15 |
| MSP25          | MSP25-00 | MSP25-24 | MSP25-3 | MSP25-7 | MSP25-15 |
| MSP26          | MSP26-00 | MSP26-24 | MSP26-3 | MSP26-7 | MSP26-15 |
| MSP27          | MSP27-00 | MSP27-24 | MSP27-3 | MSP27-7 | MSP27-15 |
| MSP28          | MSP28-00 | MSP28-24 | MSP28-3 | MSP28-7 | MSP28-15 |
| MSP29          | MSP29-00 | MSP29-24 | MSP29-3 | MSP29-7 | MSP29-15 |
| MSP30          | MSP30-00 | MSP30-24 | MSP30-3 | MSP30-7 | MSP30-15 |
4. CONCLUSION

The conclusion will be drawn from the results obtained and observations which will be observed. The conclusions will contain analytical observations between MSB and MSP. For relative oral bioavailability the maximum concentration of calcium by plotting area under curve (AUC) will be assessed between standard calcium supplement tablet, MSP and MSB. According the blood plasma concentration of serum calcium, the graph will plotted against time in all three groups. The drug with maximum area under curve will be concluded as better relative oral bioavailable.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The study will be conducted on human volunteers. The permission is obtained from the related institutional ethical committee (IEC). The approval reference number is Ref.No.MGACHRC/IEC/July-2020/64.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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