

Study Protocol

Evaluation of relative oral bioavailability of *Shankha Bhasma* prepared by two different methods in healthy volunteers: A Study Protocol

ABSTRACT

Background: *Rastarangini Shankha* (Conch Shell) *Bhasma* is one of the traditional medicines reported to be used for centuries in the treatment of calcium deficiency disorders. It proves to be a good source of calcium. But reports on its bioavailability are hardly available. This study aims to assess the methods of preparation of *Shankha Bhasma* and assess its relative oral bioavailability in healthy volunteers.

Material and Method: This study will include preparation of *Shankha Bhasma* in *Rasashastra* department. The traditional method will be giving *Putas*. Another method includes heating *Shudhha Shankha* on gas flame. The products obtained with these procedures will undergo *Bhasma Pariksha* that includes organoleptic characteristics, physicochemical parameters, microbial contamination and other relevant tests. This will be randomized single blind control study of 90 healthy volunteers (30 in each group). The data on oral bioavailability in healthy volunteers will be analyzed by using Pair and unpaired “t” Test, One way ANOVA and descriptive statistics.

Results: The relative oral bioavailability of *Shankha Bhasma* is expected to show all pharmaceutico analytical parameters within normal range and reflect significant effects on healthy volunteers.

Conclusion: Significant efficacy of this product directs a new gateway for the management of bioavailability of calcium drugs.

Keywords: Standardization, *Shankha Bhasma*, , Pharmaceutico-analytical Parameters, Bioavailability, Calcium.

INTRODUCTION

Ayurveda is known as age old well-documented Indian system of Medicine. *Rasashastra*—a popular branch of an Ayurveda pharmaceuticals, flourished during the medieval period mostly deals with therapeutic uses of minerals, metals and mercurial preparations^[1,2]. *Rasashastra* deals with the ancient pharmaceutical preparation of Ayurveda^[3]. The subject is related to the procedures like *Shodhana*, *Marana*, *Jarana* of *Rasadi Dravyas* like *Rasa*, *Maharasa*, *Uparasa*, *Sadharanarasa*, *Dhatu* and *Updhatu*^[4].

They have additional advantages over herbal drugs i.e. *Rasa-Aushadhies* are effective in smaller doses, having agreeable taste, quick action and require shorter duration of administration. Almost all the substances used in *Rasashastra* such as metals, minerals, poisons and animal origin drugs need to be transformed to make them human friendly, pharmaco-therapeutically useful and

safe. Multiple pharmaceutical processing like *Shodhana and Marana* were developed later which render toxic substances into non-toxic, more potent for elimination of disease and promoting health. *Sudhavarga* is one among them, which is considered as one of the group of drugs in the field of *Rasashastra*. It consists of many drugs like *Sudha, Shankha, Shukti, Godanti, Egg shell Mrigshringa, Khatika* etc. which content calcium compounds as a major component ^[5].

Calcium compounds were known in Ayurveda science since second centuries BC. However the method of preparing calcium compounds in therapeutic dosage form is different according to different *Acharyas*. *Shankha Bhasma* is one of such calcium supplement which is cheap and abundantly available in nature. Few clinical studies revealed clinical efficacy of *Shankha Bhasma* in calcium deficiency disorders such as osteoporosis. However considering the different methods of purification and incineration to prepare *Shankha Bhasma*, its analytical standardization and bioavailability study is still a major gap in Ayurveda. According to the various classical texts, the *Shodhan* of *Shankha Bhasma* is done in the *Amladravya*. According to the *Acharya Siddhinandan Mishra*, the *Shodhana* of *Shankha* is done in *Amladravya* mainly in *Nimbuka Swarasa, Jambir Nimbu Swarasa, Tanduladaka, Kanji* and *Jayanti Patra Swarasa*. but in his text he also mentioned that *Shodhana* of *Shankha* can be done in various *Amla dravya varga* ^[6]. *Acharya Bhavpraksha* described *Amladravya Varga* in which various drugs are mentioned having *Amla* property. So for this study *Amlika* is one of the *Amladravya* can be used for the *Shodhana* of *Shankha*. *Amlika* is easily available and cheap drug. In *Sharangdhar Samhita* under the *Swarasa Kalpana* there are three methods described to prepare *Swarasa* from drugs. As *Amlika* is considered under the hard drug. *Swarasa* is prepared by soaking the drug with 2 parts of water overnight and then filtering it through a piece of cloth.

Calcium bioavailability is considered as significant when an individual is losing or growing bone or when calcium intake is low. Various dietary and environmental factors such as protein, sodium, caffeine vitamin D, fructose and phosphorous show impact on Calcium absorption in the body. In addition, individual's genetic makeup, including the vitamin D receptor genotype, may also play a key role in calcium absorption. Supplementation with various calcium preparations is now available. However it is observed that the bioavailability of many commercial calcium products is dissimilar ^[7]. Hence, this study is planned for the bioavailability of *Shankha Bhasma* compound.

Rational of study

In today's era calcium deficiency disorders are increasing day by day. According to age dietary calcium intake is ranging from 400 to 1200 mg per day. Several modern diets failed to provide the required level of calcium. Large number of standard calcium supplements is available in market, but today's necessity is the high solubility calcium content, that could be an efficient source of calcium through dietary intakes. *Bhasma* contain Nano particles which is having higher absorption and distribution property. There is no information available carrying the bioavailability of *Shankha Bhasma* as calcium form.

There were many studies conducted regarding the preparation of *Shankha Bhasma* by doing *shodhana in amaldravya swarasa*, but *Shodhana* in *Amlika Swasaras* is not done before though this drug is freely available and economical. *Shankha Bhasma* is used in many formulations and indicated in many gastrointestinal diseases but oral bioavailability study of *Shankha bhasma* is not conducted till now. Therefore this study is planned to evaluate the oral bioavailability of *Shankha Bhasma* and compare with the Standard calcium supplement.

MATERIAL AND METHODS

Study design –This is the pharmaceutical, analytical and experimental study. Study will be conducted at Mahatma Gandhi Ayurved College, Hospital & Research Centre, Salod (H), Wardha. Present work will be conducted under following headings;

a) **Pharmaceutical study:** This study is related to drug preparation in which two different batches of *Shankha Bhasma* will be prepared to establish pharmaceutical standardization. Pharmaceutical study will be done in following steps;

I) **Procurement of Raw materials:** All raw materials required for the study will be procured from authentic reliable sources. *Kumari* (Aloe Vera) will be procured from Herbal Garden (MGAC), *Amlika* from authenticated vender. *Shankha* will be collected from well known raw material supplier from Nagpur.

II) **Authentication of Raw materials:** Raw drugs (Herbs) will be verified and authenticated by Department of Dravyaguna of MGAC & RC. *Shankha* will be authenticated by Department of Rasashastra. Raw drugs will be standardized as per A.P.I.

III) **Shodhana (Purification) procedure of *Shankha*** ^[8,9]

Shodhana of *Shankha* will be done in *Dolayantra* in *Amlika swarasa* as per described in *Sharnagadhar smahita*.

Preparation of *Amlika Swarasa*

Take one part fruit pulp of *Amlika*

↓
Soaked overnight in double quantity of portable water

↓
Next morning macerate the mixture

↓
And filter through a white clean cloth to obtain *Swarasa*

Flow diagram of *Shodhana (Purification) of Shankha*

In *Khalvayantra* *Shankha* will be made in to smaller pieces.

↓
Then *Shankha* will be tied in a white clean cloth and make a *Pottali*.

↓
It will be hanged in *Dolayantra* containing *AmlikaSwarasa* (It is a *Shodana Dravya*)
As liquidmedia is subjected for *Swedana* of one *Yama*(3 hours)

↓
Later the drug in the cloth will be washed with warm water and dried and stored as *Shuddha Shankha*.

IV) *Marana (Incineration) procedure of Shankha by Method 1 &Method 2* ^[10]

Flow diagram of *Marana (Incineration) procedure of Shankha (Method 1)*

ShuddhaShankha will be triturated it in *Khalvayantra*



Kumari(Aloe Vera) *Swarasa* will be added in *Khalva Yantra* and triturate till it becomes a fine paste

↓
Small *Chakrikas* will be prepared and dried

↓
The *Chakrikas* will be enclosed in *Sarava Samputa*

↓
The *Samputa* is sealed appropriately and dried under sun

↓
Later it will be subjected for one *Gajaputa* with dry cow dung cakes

↓
When cool on its own, the white colored *Bhasma* inside the *Samputa* will be collected

↓
It will be Triturated and stored in airtight container as *Shankha Bhasma*

V) Flow diagram of *Marana* (Incineration) procedure of *Shankha* (Method 2)

The *Shudhha Shankha* will be heated on gas flame with using Charcoal

↓
Heat till it becomes brittle

↓
Collect brittle *Shankha* and powdered in *Khalva Yantra*

↓
Stored in airtight container as *Shankha Bhasma*.

b) Analytical study:-For analytical study organoleptic characters and physicochemical parameters, microbial contamination, and other sophisticated tests like Particle size distribution analysis, SEM –EDX, AAS, FTIR, XRD, and GCMS will be done.

c) Bioavailability study: This will be randomized single blind control study of 90 healthy volunteers (30 in each group). The subject will be selected from Mahatma Gandhi Ayurveda College Hospital & Research Centre, Salod(H), Wardha.

Eligibility criteria: Volunteers between age group 20 – 40 years of male sex after physical examination and Complete blood count (CBC), Blood sugar, Liver function test, Kidney function test, Lipid profile, Blood pressure with normal values will be selected.

Interventions: Total 90 healthy volunteers divided in 3 groups (30 in each group) one standard group and other two will be study groups. For standard control group, Calcium standard supplements will be given 500mg once a day before meal and for both study groups *Shankha Bhasma* tablet prepared by (Method-1) and (Method-2) will be given 500mg once a day before meals for 15 days.

Table 1: Grouping and Posology

Group	Group Code	Supplements	Dose	Anupan	Duration	Fallow-up	Participant
Standard Control	SC	Calcium Standard supplements	500mg Once a day Before meal	Water	15 Days	After 24 Hrs, 3 rd Day, 7 th Day, 15 th Day of drug administration	30
Study Group-1	SG-1	<i>Shankha Bhasma</i> (Method-1)	500mg Once a day Before meal	Water	15 Days	After 24 Hrs, 3 rd Day, 7 th Day, 15 th Day of drug administration	30
Study Group-2	SG-2	<i>Shankha Bhasma</i> (Method-2)	500mg Once a day Before meal	Water	15 Days	After 24 Hrs, 3 rd Day, 7 th Day, 15 th Day of drug administration	30

Investigation during treatment: Complete blood count (CBC), Liver Function Test (LFT), , Kidney Function Test (KFT), Lipid profile, Blood sugar, Urine routine and microscopic, Urine calcium, Blood Sr. Calcium Level.

Criteria for discontinuing or modifying allocated interventions:

Subjects will be withdrawn from the study if any untoward incidence, features, feature of drug sensitivity or any other disease or problem arises the subjects will be offered from free treatment till problem subsides.

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

Primary outcomes: The relative oral bioavailability of *Shankha Bhasma* will be observed

Implementation: Principle investigator will allocate and enroll the patients.

Statistical analysis: Statistical analysis will be done by applying pair and unpaired “t” test, One way ANOVA and descriptive statistics.

Observation & Results:

Observations will be noted and presented in the form of tables, charts, photographs etc. The expected result of the study is that study group B and C with intervention Standard calcium supplement will be potentially more effective as a good calcium source. Patient who will take all fallow up by following *Pathya* and *Apathya* during treatment will have less chances of calcium deficiency disorders as compare to group A.

DISCUSSION

Conch is a common name that is applied to a number of different medium to large sized “sea snail “or their “shells”. The term generally applies to large sea snails that have a high spire and a

siphonal canal (comes to a point at both ends of the shell). True conch are marine Gastropoda Molluscs in the family Strombidae, specially in the genus Strombus and other closely related genera such as Eustrombus. Chemically it is Calcium Carbonate (CaCO_3). It contains mainly carbonate of calcium, iron, magnesium, sulphate, phosphate and chloride.

Medicinal plants are the major source as a medicine for the majority of the population throughout the globe. Increase in population, cost of allopathic treatment for general ailments, side effects of modern drugs and development of resistance have led to increase emphasis on the use of herbs and minerals as source of medicines and health supplements. ^[11]

However according to the modern science, each drug needs to be scientifically, pre-clinically and clinically evaluated for the global acceptance ^[12]. In medical field, the analytical studies of Ayurvedic formulations help to form the base for standardization. It is the need of the hour to generate evidence for existing literature and for reproducibility. ^[13-15] Standardization of Ayurvedic medicines on pharmaceutical and analytical level is the most important aspect before beginning research on pre-clinical and clinical ground. In this protocol two different methods of preparation will be used to make *Shankha Bhasma*. There intervention will be with standard calcium supplement. One group will receive *Shankha Bhasma* prepared by method-1 and other will receive *Shankha Bhasma* prepared by method-2 and third group will receive Standard Calcium Supplement. The assessment will be done based on subjective and objective parameters. After that data will be analyzed by using statistical test and present in the form of table and charts. ^[16-28]

CONCLUSION:

This research work on *Shankha Bhasma* will show all pharmaceutico analytical parameters within range and significant effects on healthy volunteers to evaluate its relative oral bioavailability. As the ingredients are easily available and method of preparation does not require much time and man power therefore if this preparation show significant efficacy then this will open a new gateway for the management of bioavailability of calcium drugs.

SCOPE AND IMPLICATION OF PROPOSED STUDY

1. Discovery of new chemical structure with new action will be done, if the bioavailability is more then we can use *Shankha Bhasma* instead of current calcium compound.
2. Synthetic calcium supplement is having the side effects of bloating gas, constipation, heart diseases & kidney stone, but if these *Shankha* formulations are proved to be better in bioavailability can be utilized very safely & effectively.
3. If the bioavailability of *Shankha Bhasma* will found significant then it will be used as one of the content in various Ayurvedic formulations or the separate *Shankha Bhasma* tablets can be prepared which will be useful in bone fractures, osteoporosis, healing etc.

TRANSLATORY COMPONENT

If the study is successful then this will be used in another clinical studies establishment on various calcium deficiency diseases.

NOTE:

The study highlights the efficacy of "Ayurveda" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

References:

1. Gupta KL, Chinta S, Reddy KR. Importance of Anandakanda in the history of Indian alchemy. *Bull. Ind. Inst. Hist. Med.*, Hyderabad 2006; 36: 159-66.
2. Rathi B, Rathi R, Pusadkar S. Contribution of text Rasapaddhati in the history of Indian alchemy: A review. *Journal of Indian System of Medicine*. 2019 Apr 1;7(2):70.
3. Mahulkar G, Rathi B. Pharmaceutical Standardisation of Kukkutanda Tvak Bhasma (Incinerated Egg Shell). *JOURNAL OF RESEARCH IN TRADITIONAL MEDICINE*. 2017;3(2):43-50.
4. Murty P.H.C. Rasashastra the mercurial system, Chaukhamba Sanskrit series office, Varanasi, 3rd ed-2013, p.415
5. Reddy KRC, Text book of Rasashastra, Chaukhamba Sanskrit Sansthan, 2nd edition, Varanasi, 2010, p 413
6. Mishra S. Ayurvediya Rasashastra, Chaukhamba Orientaliya, 3rd edition, Varanasi, 2003, p.574
7. Kressel G. Bioavailability and solubility of different calcium -salts as a basis for calcium enrichment of beverages, *Food and Nutrition science*, 2010, 1, p.53-58
8. Angadi R. Rasatarangini, Chaukhamba Surbharati Prakashan, 1st edition, Varanasi, 2015, 12-6-7, p.192
9. Tripathi B. Sharangdhar Samhita, Madhyam Khand, Chaukhamba Surbharati Prakashan, reprint edition, Varanasi-2019, p.85.
10. R. Rajeshwari, *Journal of Pharmacognosy and Phytochemistry*, Aloe Vera: The Miracle plant its Medicinal and Tradition uses in India JSSN 2278-4136, ZDB-Number:2668735-5 JC Journal No:8192 Volume 1, Issue 4.
11. Rathi, B. and Rathi, R., Quantitative Analysis of Medicinal plants used by the Traditional healers of Karanja block of Wardha district for treating Musculoskeletal disorders. *International Journal of Ayurvedic Medicine*, 11(2), pp.175-183
12. Chavan, R., Khan, M., Sathe, N. and Mankar, N.A., 2016. A Review: SRB Assay for Screening Anticancer Activity of Herbal drugs (in-Vitro). *International Ayurvedic Medical Journal*, 4(2), pp.66-70.
13. Rathi B, Rathi R. Pharmaceutical standardization of Bakuchi vati: A modified dosage form of Dhatriyadi Yoga, *International Journal of Research in Ayurveda and Pharmacy*, 2017;8(1);57-61
14. James SL, Castle CD, Dingels ZV, Fox JT, Hamilton EB, Liu Z, Roberts NL, Sylte DO, Bertolacci GJ, Cunningham M, Henry NJ. Estimating global injuries morbidity and mortality: methods and data used in the Global Burden of Disease 2017 study. *Injury Prevention*. 2020 Oct 1;26(Suppl 2):i125-53.
15. Kumar A, Chery L, Biswas C, Dubhashi N, Dutta P, Dua VK, Kacchap M, Kakati S, Khandeparkar A, Kour D, Mahajan SN. Malaria in South Asia: prevalence and control. *Acta tropica*. 2012 Mar 1;121(3):246-55.
16. Chole RH, Patil RN, Basak A, Palandurkar K, Bhowate R. Estimation of serum malondialdehyde in oral cancer and precancer and its association with healthy

- individuals, gender, alcohol, and tobacco abuse. *Journal of cancer research and therapeutics*. 2010 Oct 1;6(4):487.
17. Pradhan S, Madke B, Kabra P, Singh AL. Anti-inflammatory and immunomodulatory effects of antibiotics and their use in dermatology. *Indian journal of dermatology*. 2016 Sep;61(5):469.
 18. Acharya S, Shukla S, Mahajan SN, Diwan SK. Acute dengue myositis with rhabdomyolysis and acute renal failure. *Annals of Indian Academy of Neurology*. 2010 Jul;13(3):221.
 19. Gadbail AR, Chaudhary M, Patil S, Gawande M. Actual Proliferating Index and p53 protein expression as prognostic marker in odontogenic cysts. *Oral Diseases*. 2009 Oct;15(7):490-8.
 20. Prasad N, Bhatt M, Agarwal SK, Kohli HS, Gopalakrishnan N, Fernando E, Sahay M, Rajapurkar M, Chowdhary AR, Rathi M, Jeloka T. The adverse effect of COVID pandemic on the care of patients with kidney diseases in India. *Kidney international reports*. 2020 Sep 1;5(9):1545-50.
 21. Walia IS, Borle RM, Mehendiratta D, Yadav AO. Microbiology and antibiotic sensitivity of head and neck space infections of odontogenic origin. *Journal of maxillofacial and oral surgery*. 2014 Mar 1;13(1):16-21.
 22. Lohe VK, Degwekar SS, Bhowate RR, Kadu RP, Dangore SB. Evaluation of correlation of serum lipid profile in patients with oral cancer and precancer and its association with tobacco abuse. *Journal of oral pathology & medicine*. 2010 Feb;39(2):141-8.
 23. Korde S, Sridharan G, Gadbail A, Poornima V. Nitric oxide and oral cancer: A review. *Oral oncology*. 2012 Jun 1;48(6):475-83.
 24. Gondivkar SM, Gadbail AR. Gorham-Stout syndrome: a rare clinical entity and review of literature. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*. 2010 Feb 1;109(2):e41-8.
 25. Gadbail AR, Chaudhary M, Gawande M, Hande A, Sarode S, Tekade SA, Korde S, Zade P, Bhowate R, Borle R, Patil S. Oral squamous cell carcinoma in the background of oral submucous fibrosis is a distinct clinicopathological entity with better prognosis. *Journal of Oral Pathology & Medicine*. 2017 Jul;46(6):448-53.
 26. Gadre PK, Ramanojam S, Patankar A, Gadre KS. Nonvascularized bone grafting for mandibular reconstruction: myth or reality?. *Journal of Craniofacial Surgery*. 2011 Sep 1;22(5):1727-35.
 27. Sorte K, Sune P, Bhake A, Shivkumar VB, Gangane N, Basak A. Quantitative assessment of DNA damage directly in lens epithelial cells from senile cataract patients. *Molecular vision*. 2011;17:1.
 28. Basak S, Rajurkar MN, Mallick SK. Detection of *Blastocystis hominis*: a controversial human pathogen. *Parasitology research*. 2014 Jan;113(1):261-5.