



---

---

## ASSESSMENT OF ANGIOGENIC EFFECT OF SWARNA MAKSHIKA BHASMA: A CAM MODEL STUDY PROTOCOL

SURYAWANSHI N\*, GHAROTE A, TOMAR E AND KONDAR S

Department of Rasashastra & Bhaishajya Kalpana, D Y Patil deemed to be University, School  
of Ayurveda, Nerul, Navi Mumbai, India

\*Corresponding Author: Dr. Nilesh Suryawanshi: E Mail: [dr.nilsurya@gmail.com](mailto:dr.nilsurya@gmail.com)

Received 16<sup>th</sup> Nov. 2023; Revised 18<sup>th</sup> Dec. 2023; Accepted 2<sup>nd</sup> June 2024; Available online 1<sup>st</sup> April 2025

<https://doi.org/10.31032/IJBPAS/2025/14.4.8841>

### ABSTRACT

**Background:** *Swarna Makshika Bhasma*, a herbo-mineral compound is widely used in many diseases like *Pandu*, *Kamala*, *Kushtha* etc. It has specific properties like *Pittashamana* and *Raktaprasadana*. In the present study *Swarna Makshika Bhasma* will be prepared with a standardized method w.s.r. To *Rasaratnasamuchchaya* and its angiogenic effect will be tested with the Chick Chorioallantoic membrane (CAM) model. This study aims to investigate potential impact of *Swarna Makshika Bhasma* on angiogenesis and establish its effect on *Raktadhatu* using more reliable and cost-effective method like CAM.

- Objectives:**
1. To prepare *Swarna Makshika Bhasma*
  2. Physicochemical and Analytical study of *Swarna Makshika Bhasma*
  3. To assess of effect of *Swarna Makshika Bhasma* on angiogenesis
  4. To compare angiogenic potential of *Swarna Makshika Bhasma* with standard drug Progesterone

**Methodology:** Classical and contemporary literature will be reviewed and the data will be collected. *Swarna Makshika Shodhana* with *Matulunga swarasa* and *marana* with *Gandhaka* (Sulphur) will be done. Analytical tests like classical *Bhasma pariksha*, physicochemical analysis and advance analysis like XRD and SEM-EDX will be done.

**Expected Results:** The results based on pharmaceutical study, analytical study and experimental study will be observed and noted to define the conclusion.

**Keywords:** *Swarna Makshika*; Angiogenic Potential; Chick Embryo; CAM model

## 1. INTRODUCTION

### 1.1 Background

*Ayurveda* is ancient Indian system of medicine practiced widely in India, Nepal, and several other Asian Countries. In *Ayurveda*, *Aushadhi Kalpanas* (Formulations) are prepared using raw materials occurring in the nature only. *Ayurveda* emphasizes on both, preventive and curative aspects through proper life style and natural medicines.

Since ancient times, minerals and metals have been used in the various forms to treat the disease and maintain the health through the properties like *Balya*, *Rasayana* etc. [1]. *Acharya Sushruta* [2] referred to these preparations of metals as "*Ayaskruti*." Minerals and metals were seldom ever utilized prior to the *Sharangdhara* period but then it has been used widely.

*Bhasmas* provide excellent results in very less doses due to their potential [3]. These *bhasmas* are essential components of any formulations mentioned in *Rasashastra*, a branch of *Ayurveda*.

*Swarna Makshika Bhasma* meets every requirement needed for the purpose of this study. Additionally, it is said to be, *Rasayana*, *Pandughna*, *Kushthaghna*, *Raktagata pitta Shaman* [4] and has a highly special feature called *Raktaprasadana*, which strengthens the selection criterion.

The vascular membrane known as the Chorioallantoic membrane, or CAM is

present in the eggs of certain amniotes, including birds and reptiles.

As one can see the function of streamlets and canals to maintain a field or garden moist and productive, the blood vessels nourish the body of an organism in the same way through their contractibility and expansibility according to *Sushruta*. This reference describes the vital role of the blood vessels are in sustaining the healthy life.

A literature search is a necessary step in every new study. A comprehensive literature search will be conducted for this study. The literature from *Samhitas*, the Internet, journals, and other sources will be searched to get thorough information. The literature review has proven to be beneficial in providing comprehensive understanding of the a forementioned principles. It is also beneficial to select the quality raw material considering its characteristics according to *Rasashastra* and other modern sciences like Metallurgy, Physics, and chemistry. Furthermore, it also helps to define the processes to be carried out on the raw material to prepare the final product.

The detailed study of *Swarna Makshika*, *Matulunga*, *Gandhaka* (Sulphur), *Shodhana* procedure, *Marana* procedure and *Putra* will be discussed in the literature review in accordance with textual references. A review of the literature will also comprise

the angiogenic effect of an experimental investigation that uses a CAM Model [5-9].

### 1.2 Rational justification

Numerous artificial medications and substances have the ability to promote wound healing and induce angiogenic responses in fibro proliferative reactions.

The CAM model is inexpensive and easy to evaluate using chick embryos, it provides the opportunity to examine drug delivery methods with multiple screenings before moving on to more costly and legally-required mammalian models.

Chick embryos has a high potential for use in pharmaceutical research in areas such as angiogenic treatments for conditions like diabetic foot, myocardial infarction and wound healing. Till date the angiogenic or anti angiogenic potential of many modern drugs has been tested by various means. *Ayurveda* provides a huge range of formulations which has proven potential effect on *Raktadhatu* (Blood) and can exhibit certain effect on vasculature. This is a small effort to explore the angiogenic effect of *Ayurveda* medicine using modern methodologies.

## 2. MATERIALS AND METHODS

### 2.1 Aims and objectives

#### -Aim

To study the angiogenic potential of *Swarna Makshika Bhasma* using CAM model

#### -Objectives

1. To prepare *Swarna Makshika Bhasma* according to *Rasaratnasamuchchaya*
2. Physicochemical and Analytical study of *Swarna Makshika Bhasma*
3. To explore the angiogenic potential of *Swarna Makshika bhasma* using the chicken chorioallantoic membrane (CAM) model.
4. To compare Angiogenic potential of *Swarna Makshika bhasma* with standard drug progesterone

### Hypothesis

**Null Hypothesis:** There is comparatively no significant difference in angiogenic potential of *Swarna Makshika Bhasma* and progesterone.

**Alternative Hypothesis:** There is comparatively significant difference in angiogenic potential of *Swarna Makshika Bhasma* and progesterone.

### 2.2- Materials and methods for study

- A) Pharmaceutical
- B) Analytical
- C) Experimental

#### A) Pharmaceutical

##### Materials:

**Drugs-** Raw *Swarna Makshika*, *Matulunga*, *Gandhaka* (Sulphur)

**Pharmaceutical Instruments** - *Khalva Yantra*, pan, Mixer, Sieves, Steel Vessels, Cotton cloths, Measuring Cylinders, *Sharava* (Clay Saucer), Electric Muffle Furnace etc.

##### Methodology:

a) Procurement and Authentication of Raw *Swarna Makshika*

b) *Shodhana* of *Swarna Makshika* [10]

The Raw *Swarna Makshika* will be procured from authentic sources. The *Shodhana* procedure of Raw *Swarna Makshika* will be done according to *Rasaratnasamuchchaya*. The powdered *Swarna Makshika* will be roasted in *Matulunga swarasa*. This roasting procedure will be carried out till it achieves reddish black colour.

c) *Marana* of *Swarna Makshika* [11]

*Swarna Makshika* levigation with *Matulunga swarasa* will be done and it will be incinerated with *shodhit Gandhaka* (Sulphur) in Electric Muffle Furnace with the heating pattern like Varaha Puta. The incineration will be repeated till it achieves *bhasma siddhi lakshan*.

## B) Analytical

The prepared *Swarna Makshika Bhasma* will be assessed utilizing a variety of analytical methods, including XRD, SEM-EDX and traditional Ayurvedic methods.

## C) Experimental

### Materials:

The white LEGHORN chicken breed's viable eggs will be chosen for the experimental investigation. Thirty-two zero-day eggs of a fertile hen. (White chick)

Instruments and equipment:

1. Trays for eggs
2. Cotton, scissors, pencil, and spirit.
3. The incubator

4. Thermocouple
5. Hygrometer (for humidity measurement)
6. 2 ml syringe with needle (50 needles)
7. Dental drill machine with variety of cutting instruments (diamond disc, mandrel for disc)
8. Blunt forceps and finely pointed forceps
9. Scotch "Magic" adhesive tape
10. Normal Saline pack
11. Egg Candling instrument
12. A matchbox and candles
13. Beaker, stirrer, pipette, Petri dish, and wax.
14. A Progesterone Capsule

### Methodology:

As the investigation progresses, the most crucial component will be an experimental study that will last for 14 days. The temperature and humidity will play a crucial role in this experimental study, which will be carried out with the utmost care because the chick embryo will undergo significant changes every day.

For Standard CAM Model Protocol [12,13] following Steps should be followed:

- a. Incubation of the fertilized eggs will take place at 37<sup>0</sup>C with 60–70% relative humidity.
- b. On Embryonic Development Day (EDD), 4–5-day old embryos will be used for testing.

c. After that, *Swarna Makshika Bhasma* will be placed on CAM surface on day 8.

d. A standard drug progesterone will be applied on CAM surface on day 8.

Table 1: Materials and Methods

S. No.	A  Pharmaceutical Study	Sr. No	B  Analytical Study	Sr. No	C  Experimental Study
1.	Procurement and Authentication	1.	<i>Bhasma Pariksha</i> acc. to <i>Rasashastra</i>	1.	Procurement of Materials
2.	<i>Shodhana</i> of 1. <i>Swarna Makshika</i> 2. <i>Gandhaka</i> (Sulphur)	2.	Physico chemical tests	2.	Chorioallantoic Membrane (CAM) study
3.	Marana of <i>Swarna Makshika</i> with <i>Matulunga Swarasa</i> and <i>Gandhaka</i>	3.	Advanced Analytical tests		

Table 2: Different tests for Bhasma

S. No.	A  <i>Bhasma Pariksha</i>	B  Physico-Chemical tests	C  Advance Analytical tests
1.	<i>Rekha poornatva</i>	Moisture Content	XRD
2.	<i>Varitaratva</i>	Total Ash	SEM-EDX
3.	<i>Uttam</i>	Acid Insoluble Ash	
4.	<i>Nirutthatva</i>	Water Soluble Ash	
5.	<i>Apoonarbhavatva</i>	Specific Gravity	
6.	<i>Amla</i>	pH	
7.	<i>Nischandratva</i>		

Table 3: The Road map of Experimental Study

Embryonic Development Day (EDD)	Procedure	Action
1	Cleaning of egg transfer to incubator	Discard cracked egg
2	Identification of fertilized egg using candling method	Observe and Mark the unfertilized egg
3	Creating window	Remove 2ml of albumin removed from narrow end of the egg
4	Observation of mortality	Discard dead embryo
5,6,7	Observation of mortality	Discard dead embryo
8	Loading the drug samples to be tested on CAM	Only viable eggs will be loaded with drug samples
9,10,11	Keep the egg undisturbed for these 3 days	Keep Observing without disturbing eggs
12	Observation of angiogenesis and mortality	Discard dead embryo
13	Observation of angiogenesis and mortality	Discard dead embryo
14	Observation of angiogenesis and mortality	Discard dead embryo

### 3. ANTICIPATED RESULTS

The observations during each and every step of *shodhan* and *marana* of *Swarna Makshika* will be documented.

*Swarna Makshika Bhasma* samples will undergo a pharmaceutical and analytical examination, and the results will be derived based on the recorded data.

### 4. DISCUSSION

As per the title “Assessment of Angiogenic effect of *Swarna Makshika Bhasma*: a CAM model study” the study is divided into three different sections such as Preparation of *Swarna Makshika Bhasma*, its Physico-chemical and Advance Analysis and assessment of angiogenic effect. The CAM model will be used to study angiogenic effect provides highly vascular membrane

surface for the application of trial drug. There one can observe the rapid embryonic development of chick embryo with naked eyes. This is the only method that provides an opportunity to observe embryonic development and the impact of medicines on vascularization of developing embryo, so closely. The angiogenesis and anti-angiogenesis both can be effectively studied with this simple and cost-effective method of drug testing. The incubation period of chick embryo is of 21 day and the neovascularization in the embryo begins at day 7 which gives a larger time to the researcher to study the effect of drug.

### 5. CONCLUSION

1. The preparation of Swarna Makshika Bhasma using Electric Muffle furnace (EMF) is rarely performed procedure.
2. This study will be an attempt to standardize the procedure for preparation of *Swarna Makshika Bhasma* using EMF.
3. Qualitative and Quantitative analysis of *Swarna Makshika Bhasma* will be done and it will be helpful for drug standardization.
4. Photographic and histopathological methods will be used to monitor angiogenic effect of *Swarna Makshika Bhasma*
5. Compatibility of CAM model with Ayurveda Medicines will be also tested through this research work.

Establishment of *Swarna Makshika Bhasma* as a therapeutic angiogenic medication for conditions such diabetic foot ulcers, anemia, ophthalmic disorders, ischemic heart disease, alopecia, and chronic wounds can be studied through Clinical trials.

### 6. REFERENCES

- [1] Charak Samhita of Agnivesha with 'Charaka Chandrika' Hindi commentary by Dr. Brahmanand Tripathi, Chaukhamba Surabharati Prakashan, Varanasi, Edition 2012, Chikitsasthana Prathamodhyaya (1<sup>st</sup> chapter), Trutiya pada (3<sup>rd</sup> section), shloka 15-23
- [2] Sushruta Samhita of Maharshi Sushruta with 'Ayurved Tattvasandeeepika' Hindi Commentary by Dr. Ambikadatta shastri, Chaukhmbha sanskrit sansthan, Varanasi, Edition 2012, Part 1, Chikitsasthana, Mahakushthachikitsa chapter 10, shloka 11, page no. 72
- [3] Rasaratna Samuchhaya of Shri Vagbhatacharya with 'Rasaprabha' Hindi commentary by Vd. Indradev Tripathi, Chaukhamba Sanskrit Pratishtan, Varanasi, Reprint edition 2012, Ashtavinshodhyaya (Chapter 28), shlok 1, page 371
- [4] Ayurved Prakasha of Shree Madhav Upadhyaya with 'Arthavidyotini' Hindi commentary by Vd.

- Gulrajsharma Mishra,Chaukhambha Bharati academy, Varanasi, Reprint edition 1999, chapter 4, shlok 7, page 410
- [5] Ribatti D. The Chick Embryo Chorioallantoic Membrane as an In Vivo Assay to Study Antiangiogenesis. *Pharmaceuticals (Basel)* 2010;3: 482–513.
- [6] Daniel V, Daniel K. Perception of Nurses' Work in Psychiatric Clinic. *Clinical Medicine Insights.* 2020;1(1):27-33.  
Available:<https://doi.org/10.52845/CMI/2020v1i1a5>
- [7] Ribatti D. The chick embryo chorioallantoic membrane as a model for tumor biology. *Exp. Cell Res.* 2014;328:314–324.
- [8] Liu M, Xie S, Zhou J. Use of animal models for the imaging and quantification of angiogenesis. *Exp. Anim.* 2018;67:1–6.
- [9] Ribatti D. The chick embryo chorioallantoic membrane (CAM). A multifaceted experimental model. *Mech. Dev.* 2016;141:70–77
- [10] Rasaratna Samuchhaya of Shri Vagbhatacharya with 'Rasaprabha' Hindi commentary by Vd. Indradev Tripathi, Chaukhamba Sanskrit Pratishthan, Varanasi, Reprint edition 2012, Dwitiyodhyaya (Chapter 2), shlok 78, page 17
- [11] Rasaratna Samuchhaya of Shri Vagbhatacharya with 'Rasaprabha' Hindi commentary by Vd. Indradev Tripathi, Chaukhamba Sanskrit Pratishthan, Varanasi, Reprint edition 2012, Dwitiyodhyaya (Chapter 2), shlok 79, page 17
- [12] Vargas, A., & Zeisser-Labouebe, M. (2007, August 16). Advanced Drug Delivery Reviews. Retrieved April 20, 2007, from <http://www.elsevier.com/locate/address>; The Chick embryo and its chorioallantoic membrane (CAM) for the in vivo evolution of drug delivery systems
- [13] Murray, J. C. (n.d.). Angiogenesis Protocol; *Methods in molecular medicine*. Chapter no. 9; Page no. 101-109