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## **In Vivo Studies of the Angiogenic Potential of Mandur Bhasma**

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**Abstract**--Background: Mandur Bhasma is a herbo-mineral compound. It is prepared by Putapaka method. It is described as Raktasanjanan. In the current study, Mandur Bhasma was prepared with a standardized method w.s.r to Rasatarangini and an experimental study was done to observe the Angiogenic property of Mandur Bhasma. The current study will analyze angiogenic potential of Mandur Bhasma using chick CAM model. This research is intended to study the possible role of Mandur Bhasma on angiogenesis and establishing properties of Mandur Bhasma as an angiogenic by newer means. The experimental study inside the egg shell will be carried out on a membrane known as “chorioallantoic membrane”. Objectives: To Prepare Mandur Bhasma, Physicochemical and Analytical study of Mandur Bhasma, To verify the angiogenic potential of Mandur bhasma using the chicken chorioallantoic membrane (CAM) model. To compare Angeogenic potential of Mandur bhasma with standard drug progesterone. Methodology: Relevant classical literature regarding

Mandur will be reviewed and the data will be collected. Mandur Shodhan with Gomutra and Mandur Maran with Triphala decoction will be done. Analytical Study like Organoleptic Test for Rasa, Gandha, Varna, Sparsha, Physicochemical Tests and other analytical test like ICP-AES /ICPMS, XRD structure of Bhasma, EDAX-NANO Particle Size will be done.

**Keywords**---angiogenic potential, CAM model, chick embryo, Mandur Bhasma.

## Introduction

*Ayurveda* mentions the use of raw materials from natural sources for preparations of different *Aushadhi Kalpanas* which are useful as preventive measures as well in the ailments. Metals are known as *Rasayana*<sup>1</sup> and as therapeutic agents since ancient times *Acharya Sushruta*<sup>2</sup> mentioned the term “*Ayaskrut*” for such metals preparations. Till the period of *Sharangdhar* metals and minerals were rarely used. But during this era the graph went high. *Bhasmas*<sup>3</sup> are an integral part of the preparation as they are considered to be the most potent form of administering these metals and minerals with good results in extremely small dosages.

*Mandur Bhasma*<sup>4,5</sup> satisfies all the properties as required for this study. It also has a very unique property i.e. the *RAKTA SANJANANA* property and it is said to be *Artavajanana* which made the selection criterion stronger. The Chorioallantoic membrane also called the chorioallantios or abbreviated to CAM- is a vascular membrane found in eggs of some amniotes such as birds and reptiles. According to *Sushruta (Su. Sha. 7/3-5)*, the blood vessels by their contractibility and expansibility sustain and nourish the organism in the same manner as streamlets and canals serve to keep a field or garden moist and fruitful. Here we can understand that how the blood vessels are important in nourishment the ailments.

To conduct any new study literature search is essential. For this study, literary search will be carried out on all ground. Literature from *Samhitas*, Internet and journal etc. will be explored to acquire from all the sources. Drug review of *Mandur, Triphala, Gomutra* and *Shodhan, Marana, Puta*<sup>6-9</sup> will be explained in review of literature according to textual references. Experimental study includes a CAM Model<sup>10-15</sup> and its angiogenesis effect is explained in review of literature. The review of literature has been helpful to understand in detailed information of the above concepts, the properties and characteristics of the raw materials. It also helps to select the raw materials and the processes to be carried out on them.

## Rational justification

There are many synthetic drugs and compounds which possess the angiogenic and wound healing properties of fibro proliferative responses. The CAM model being low cost as well as simple to assess with chick embryo offers the possibility of performing multiple screenings of drug delivery systems before it can be used

on mammalian models, which are more expensive and need approval of authorities.

There is a great opportunity for using chick embryos in the areas of growing interest in pharmaceutical research for such angiogenic therapies such as in Myocardial Infarction, Wound healing, diabetic foot, etc. The visibility, accessibility, and rapid developmental growth of the chorioallantoic membrane (CAM) offer clear advantages to study and manipulate vascular functions.

### **Aims and objectives**

Aim: - in vivo study of the angiogenic potential of *Mandur Bhasma*.

### **Objectives**

- To Prepare *Mandur Bhasma*
- Physicochemical and Analytical study of *Mandur Bhasma*
- To verify the angiogenic potential of *Mandur bhasma* using the chicken chorioallantoic membrane (CAM) model.
- To compare Angeogenic potential of *Mandur bhasma* with standard drug progesterone

### **Hypothesis**

- Null Hypothesis: There is no significant difference between *Mandur Bhasma* and progesterone in angiogenic potential
- Alternative Hypothesis: There is significant difference between *Mandur Bhasma* and progesterone in angiogenic potential

### **Materials and Methods**

- Pharmaceutical
- Analytical
- Experimental

Table 1  
Materials and methods

Materials and Methods					
Sr. No.	A] Pharmaceutical study	Sr. No.	B] Analytical study	Sr. No.	C] Experimental study
1.	Procurement and authentication	1.	Physico chemical Tests	1.	Procurement of Material
2.	Preparation of Triphala Kwath	2.	Advance Analysis Tests	2.	Chorioallantoic membrane(CAM Model)
3.	Preparation	o 3.	Bhasma Pariksha		

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1. Mandur Bhasma  
Shodhan (Swedana)
  2. Bhavana of Triphala  
Kwath to Shodhit  
Mandur
  3. Marana Samskara
- 

- Pharmaceutical  
Drugs- Raw *Mandur*, Triphala decoction  
Pharmaceutical Instruments - *Khalva Yantra*, Mixer, Sieves, Steel  
Vessels, Cotton cloths, Measuring Cylinders etc.

### Methodology

- Procurement and Authentication of *Mandur*
  - *Shodhan of Mandur*  
Processing of the Mandura will be done according to the process listed in “RasaTarangini” and AFI. Raw materials will be obtained from local market. Coarse powdered Mandura will be heated up to red-hot stage (630°C temp) and quenched in cow's urine. This purification process will be repeated for seven times.
  - *Maran of Mandur*
- To standardize the CAM activity under laboratory conditions (Angelica Vargas, 2007)
  - The fertilized eggs will be incubated at 37°C under 60-70% relative humidity.
  - Then 4 to 5 days old embryos will be used for experimentation, Embryonic Development Day (EDD).
  - Then *Mandur bhasma* will be applied on the CAM surface.
  - Standard drug will be applied on the CAM surface.

### Analytical

Table 2  
Analytical

A] <i>Bhasma Pariksha</i>	B] Physico-chemical tests	C] Advance Analysis tests
1) <i>Varitara Bhasma</i>	1) Moisture Content (LOD)	1) ICP-AES
2) <i>Rekhapuranatwa Bhasma</i>	2) Total Ash	2) XRD
3) <i>Uttama Bhasma</i>	3) Acid Insoluble Ash	3) FEG-SEM
4) <i>Niramlatwa</i>	4) Water Insoluble Ash	
	5) Specific Gravity	
	6) Elemental assay	
	7) pH	

The prepared Mandur bhasma will be characterized by traditional Ayurvedic method and by using various analytical techniques such as EDAX-SEM, XRD,

FTIR and BET. The NPST test also performed for confirmation of complete synthesis of Bhasma.

### **Experimental**

As the study goes the very important part of study i.e. experimental study which will be carried out for 14 days. This study will be done with utmost care, the temperature and the humidity will be very important factor in this experimental study, because major changes will be seen in the chick embryo on each new day. These changes and other experimental studies will be explained in detail.

- Procurement of materials
- Chorioallantoic membrane (CAM Model)

### **Procurement of materials**

For the experimental study, the fertile eggs will be selected which were of white LEGHORN chicken breed. All materials will be procured from authentic suppliers. After procurement departmental identification and selection will be done. The details are as follows-

Table 3  
Procurement of Raw material

Sr. No.	Raw material	Quantity	Supplier
1.	Fertile hen's zero day eggs. (white chick)	32	Aarey Polatary farm, Goregaon, Mumbai
2.	Incubator	1	D.Y. Patil Medical College, Nerul, Navi Mumbai
3.	Dental drill machine	1 machine	D.Y. Patil Medical College, Nerul, Navi Mumbai
4.	Scotch "Magic" Adhesive tape.	1 packet	Stationary, Thane
5.	Normal Saline (NS)	1 pint	Medical store, D.Y. Patil Medical College, Nerul, Navi Mumbai.

### **Chorioallantoic membrane (CAM Model)**

#### Materials

- Fertile hen's zero day eggs. (white chick)
- Egg trays
- Spirit and cotton, scissors, pencil.
- Incubator
- Thermometer
- Hygrometer (To measure the Humidity)
- 2 ml syringe with needle (50 needles)
- Dental drill machine with selection of cutting tools (mandrel for disc, diamond disc)
- Fine pointed forceps, blunt forceps

- Scotch “Magic” adhesive tape.
- Normal saline.
- Egg candling instrument.
- Candles and matchbox
- Wax, Pipette, Petri dish, stirrer, beaker.
- Sample bottle
- Progesterone capsule

Table 4  
How experimental study will be done

EMBROYNIC DEVELOPMENT DAY (EDD)	PROCEDURE	ACTION AFTER PROCEDURE
1	Cleaning of egg transfer to incubater	Discard cracked egg
2	Identification of fertilized egg candling method	Mark the unfertilized gg
3	Creating window	2ml of albumin removed from narrow end
4	Observation of mortality	Discard dead embryo
5,6,7	Observation of mortality	Discard dead embryo
7	Observation of mortality	Discard dead embryo
8	Loading test sample	Only viable egg are loaded with sample
9,10,11	Keep the egg undisturbed for 3 days	----
12	Observation of angeogenesis and mortality	Discard dead embryo
13	Observation of angeogenesis and mortality	Discard dead embryo
14	Observation of angeogenesis and mortality	Discard dead embryo

### Expected Results

- Observation will be recorded during the practical work of Shodhan and preparation of *Mandur Bhasma*
- Pharmaceutical Analytical study of *Mandur Bhasma* samples will be recorded
- Result will be drawn on the basis of data recorded.

### Discussion

As the title goes “In-Vivo Angiogenic effect of *Mandur Bhasma* Using Chorio allantoic membrane” but this study is divided into three parts such as *Mandur Bhasma*, Angiogenic effect and chorioallantoic membrane; but still title talks about IN- VIVO. In-Vivo- So in-vivo is a Latin word which means “within the living” .In this topic the word in- vivo is used for the study “inside the egg shell”.

The study inside the egg shell will be carried out on a membrane known as “chorioallantoic membrane”. The chorioallantoic membrane will come in the third part of our study. This membrane is highly vascular formed by combination of chorio and allantos. The chick embryo incubation period is 21 days. On 21 day eggs are hatched. In this 21 days from zero day till the eggs are hatched many physical changes will take place. One such change considering our study will take place on the 7<sup>th</sup> day i.e. the neovascularization. After the neovascularization, the second part of our study i.e. Angiogenesis will commence.

### **Conclusion**

- Weight of *Mandur bhasma* and highest temperature at the time of put procedure will be recorded,
- Qualitative analysis of *Mandur Bhasma* will be done
- Viability and mortality of eggs will be recorded
- Advantages of CAM model over other *in vivo* models used to study angiogenesis and vascular biology will be observed
- Photography and histopathological investigations will be done to observe Angiogenic effect of *Mandur Bhasma*

### **Further scope of study**

*Mandur Bhasma* can be studied for clinical trials as the therapeutic angiogenic drug in diseases such as Diabetic foot ulcers, Ischemic heart disease, Alopecia, chronic wound.

Conflict of interest: Nil

Source of funding: Nil

Ethical clearance: Taken from institutional ethics committee

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