In-Vivo Studies of the Angiogenic Potential of Mandur Bhasma

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Authors’ contributions
This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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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:
1. To Prepare Mandur Bhasma

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2. Physicochemical and Analytical study of Mandur Bhasma
3. To verify the angiogenic potential of Mandur bhasma using the chicken chorioallantoic membrane (CAM) model.
4. 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.

Expected Results: Changes will be observed in objective outcomes.

Conclusion: Conclusion will be drawn by suitably analyzing data.

Keywords: Angiogenic potential; Mandur bhasma; chick embryo; CAM model.

1. INTRODUCTION

1.1 Background/Rationale

Ayurveda mentions the use of raw materials from natural sources for preparations of different Aushadhi Kalpanas which are useful as preventive measures as well as in the ailments. Metals are known as Rasayana [1] and as therapeutic agents since ancient times Acharya Sushruta [2] mentioned the term “Ayaskruti” for such metals preparations. Till the period of Sharangdhar metals and minerals were rarely used. But during this era the graph went high.

Bhasmas [3] 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 [4,5] 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 chorioallantoic 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 [6-9] will be explained in review of literature according to textual references. Experimental study includes a CAM Model [10-15] 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 [16-19]

2. RATIONAL JUSTIFICATION

There are many synthetic drugs and compounds which possesses 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.

3. AIMS AND OBJECTIVES

3.1 Aim

In vivo study of the angiogenic potential of Mandur Bhasma.
3.2 Objectives

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

3.3 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

4. MATERIALS AND METHODS

A) Pharmaceutical

B) Analytical

C) Experimental

4.1 Methodology

A-1) 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**

A-2) To standardize the CAM activity under laboratory conditions (Angelica Vargas, 2007)

a. The fertilized eggs will be incubated at 370C under 60-70% relative humidity.

b. Then 4 to 5 days old embryos will be used for experimentation, Embryonic Development Day (EDD).

c. Then Mandur bhasma will be applied on the CAM surface.

d. Standard drug will be applied on the CAM surface.

B) Analytical

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.

C) 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.

A] Procurement of materials
B] Chorioallantoic membrane (CAM Model)

A] 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-

B] Chorioallantoic Membrane (CAM Model)

4.2 Materials

1. Fertile hen’s zero day eggs. (white chick)
2. Egg trays
3. Spirit and cotton, scissors, pencil.
4. Incubator
5. Thermometer
6. Hygrometer (To measure the Humidity)
7. 2 ml syringe with needle (50 needles)
8. Dental drill machine with selection of cutting tools (mandrel for disc, diamond disc)
9. Fine pointed forceps, blunt forceps
10. Scotch “Magic” adhesive tape.
11. Normal saline.
12. Egg candling instrument.
13. Candles and matchbox
14. Wax, Pipette, Petri dish, stirrer, beaker.
15. Sample bottle
16. Progesterone capsule

5. 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.

Table 1. 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(CAMModel) |
| 3.      | Preparation  
1. Mandur Bhasma Shodhan (Swedana)  
2. Bhavana of Triphala Kwath to Shodhit Mandur  
3. Marana Samskara | 3.      | Bhasma Pariksha |                      |          |

Table 2.

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

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 | 1machine | D.Y.Patil Medical College, Nerul, Navi Mumbai |
| 4.      | Scotch “Magic” Adhesive tape. | 1packet | Stationary, Thane |
| 5.      | Normal Saline (NS) | 1pint | Medical store, D.Y.Patil Medical College, Nerul, Navi Mumbai. |
Table 4. How experimental study will be done

| Embryonic Development Day (EDD) | Procedure | Action after Procedure |
|-------------------------------|-----------|------------------------|
| 1                             | Cleaning of egg transfer to incubator | Discard cracked egg |
| 2                             | Identification of fertilized egg candling method | Mark the unfertilized egg |
| 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 angiogenesis and mortality | Discard dead embryo |
| 13                            | Observation of angiogenesis and mortality | Discard dead embryo |
| 14                            | Observation of angiogenesis and mortality | Discard dead embryo |

6. DISCUSSION

As the title goes “In-Vivo Angiogenic effect of Mandur Bhasma Using Chorioallantoic 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 7th day i.e. the neovascularization. After the neovascularization, the second part of our study i.e. Angiogenesis will commence.

7. CONCLUSION

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

8. 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, Alopacia, chronic wound.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical clearance taken from institutional ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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