A Research Protocol for Validation of ‘Purishdhara Kala Sa Eva Ashtidhara Kala’ Using Unripe Bael Fruit (Aegle Marmelos Linn.) in Osteoporosis

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ABSTRACT

The Kala is the structure that intervenes between the Dhatus (the vital tissues) and their Ashayas (the structure that holds the particular tissue). Kala is seven in number. As per the commentary by Acharya Dalhana, the Purishdhara Kala is the Asthidhara Kala which is described in visha-vegantara in kalpashtha. This relationship has also clinical importance in Ayurvedic treatment. This relation has not yet been studied. As Kala is the structure intervening Dhatus and it’s Ashaya, the Asthidhatu (bones) is directly related to Asthidhara Kala, whereas the Purishdhara Kala is situated in the large intestine. A Research Protocol for Validation of Purishdhara Kala Sa Eva Ashtidhara Kala Using Unripe Bael Fruit (Aegle Marmelos Linn.) In Osteoporosis. In the present study, unripe Bael fruit (Aegle Marmelos Linn.) has been taken into account as an intervention because the Bael fruit is considered as the best remedy for gastrointestinal disorders, particularly the diseases of the large intestine. During this preclinical study, osteoporosis (OP) will be induced in Sprague Dawley rats with steroid. Unripe Bael fruit powder mixed with distilled water will be administered to the rats orally and transrectal. At the end of the study, the changes will be assessed on the following parameters. The histological changes in the colon and periosteum will be studied with the help of expert histopathologists. After resection of the portion of the colon, the mucosal surface will be graded with the help of a magnifying lens. A piece of the colon will be fixed in phosphate-buffered formaldehyde, embedded in paraffin, sectioned (4 μm thick), and stained with hematoxylin and eosin. Each sample will be observed and evaluated under a light microscope by two independent observers. We anticipate that there will be an improvement in markers for osteoporosis after administration of unripe Bael fruit. Hence here is an effort to determine the protocol for the assessment of the relationship between Purishadhara and Asthidhara Kala.

Key Words: Purishdhara kala, Asthidhara kala, Bael fruit, Eagle Marmelos L., Osteoporosis

INTRODUCTION

Kala is well-defined as “Dhavatshayantar Maryada” in classical Ayurvedic text. Kalais stratum or sheath existing between Dhatu and Ashaya. Acharya Sushrut has described Kala Sharir in Sharirithana of Sushrut Samhita. Among the seven Kalas, Purishdhara Kalais the fifth Kala. Pakwashaya is the location of Purishdhara Kala. Purishdhara Kalacovers Pakwashaya internally from Unduka (Cecum) to Guda (anal canal). The Kalais located in the large intestine. It is the internal layer of the colon. Separation of Ghana mala (faeces in solid form) from the Drava mala (faeces in liquid form) is completed by the Purishdhara Kala.

According to Acharya Dalhana, Purishdhara Kalais the same as Asthidhara Kala. The statement by Acharya Dalhana regarding Purishdhara Kala, gives us a new vision to study Purishdhara Kala again and explore the possibilities of physiology, pathology of the Kalais a new perspective. The Purishdhara Kala provides us with a new pathway to the area of orthopaedics. It can give us a new approach for the treatments of diseases like osteoporosis. Osteoporosis is broadly defined as “a progressive systemic skeletal disease characterized by a low bone density mass and microarchitectural deterioration of bone tissue and a consequent increase in bone fragility and susceptibility to fracture.”

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ISSN: 2231-2196 (Print) ISSN: 0975-5241 (Online)
Received: 05.09.2020 Revised: 18.10.2020 Accepted: 15.11.2020 Published: 16.02.2021
Previous studies in humans and animals point out that ageing is related to the increase in reactive free radicals are the principal factors for deterioration of bone strength and mass. The risk factors identified along with reduced bone density include heredity or maternal family history of hip fracture, hormonal disturbances, weight or height reduction, sedentary lifestyle, low body mass index, smoking and deficiencies in calcium. Osteoporosis may also be caused by other diseases such as rheumatic joint disorders or gastrointestinal disease as well as prolonged corticosteroid therapy.

The present study has been undertaken to analyse the possibility of Purishdhara Kala as principal factor responsible for the metabolism in bone tissue and thus to define the role of Purishdhara Kalas a route of drug administration to reach the bones more effectively.

Aegle Marmelos is one of the prominent medicinal plants in Ayurveda. It is also known as Bael in local language and is used in various traditional medicines in India, Nepal, Sri Lanka, Bangladesh, and Myanmar. The common English names used to refer A. Marmelos are viz. wood apple, Bengal quince, golden apple, Indian quince, holy fruit, and stone apple. A sweet drink made from the pulp of ripe fruit is intended to be protective against bacillary dysentery. Bael’s unripe fruits are stated to help treat diarrhoea, dysentery with constipation spells, and stomach ache. Extract of Wood apple unripe fruit displays efficacy against inflammatory bowel disease (IBD) in Wistar albino rats. A dose-dependent decrease in intestinal inflammation occurs following the treatment with unripe fruit extract. Likewise, significant protection in mast cell degranulation is observed in acetic acid and indomethacin-induced IBD models. Similarly, ethanolic extracts of A. Marmelos ripe fruit demonstrate the diuretic effect in rats.

Many researchers have studied Purishdhara Kala as Asthidhara Kal as a literary study. Few clinical studies can be found, but it is assessed on subjective parameters. The validation of Purishdhara Kala as Asthidhara Kalas has not been undertaken on objective parameters. Supportive objective parameters e.g. biochemical parameters, and BMD are not found to be studied. If the study could validate Acharya Dalhana’s statement about Purishdhara Kala as Asthidhara Kalai could be a breakthrough in the treatment of osteoporosis. Bael is a native of India and is easily available in India. It is consumed as an ingredient of local beverages by the Indians. Bael can prove to be a safe and cheaper source of medicine for bone disorders.

To validate the Purishdhara Kala as the Asthidhara Kala, using unripe Bael fruit (Aegle marmelos L.) as an anti-osteoporotic treatment in rats with induced osteoporosis

1. To study Ayurvediya concepts of Purishdhara Kala and AsthidharaKala.

2. To study the effect of administration of unripe Bael fruit (Aegle marmelos L.) on Asthidhara Kalai osteoporotic rats with oral and transrectal route

3. To study the effect of unripe Bael fruit (Aegle marmelos L.) on biochemical parameters selected the for study

4. To study the effect of unripe Bael fruit (Aegle marmelos L.) on bone mineral density.

5. To study the effect of unripe Bael fruit (Aegle marmelos L.) on histology of the colon and periosteum in the control group and the experimental group.

6. Comparison of effect on biochemical parameters, bone mineral density and histological changes in colon and periosteum in the control group and experimental group.

**MATERIALS AND METHODS**

**Settings:**
The study will be conducted in the Department of Racha-na Shairi at Mahatma Gandhi Ayurved College Hospital and Research Centre, (Datta Meghe Institute of Medical Sciences) in collaboration with Wardha Jawaharlal Nehru Medical College, Sawangi (Meghe), and DMAMCH&RC Nagpur. As per the necessity, the study will be conducted as per certified centre recognized by DMIMS of national repute.

**Research design:**
Study design - Randomised disease control animal study in Sprague Dawley rats. The data collected will be qualitative as well as quantitative.

Qualitative data will be acquired from the histological study of the samples of the colon. Quantitative data will be collected in the form of biochemical parameters and values of the bone mineral density.

**Duration of the study: 3 years**
Participants and sources: Total of 24 Sprague-Dawley rats will be recruited. 4 groups will be prepared with 6 Sprague-Dawley rats per group. Animals will be housed under a standard condition of temperature (22°C ± 2°C), the relative humidity of 55 ± 5% and 12 h light/dark cycles. The animals will be given standard pellet diet and water.

**Preparation of the Material:**
Unripe fruit will be collected from the botanical garden of MGAC, Wardha in the season of availability of the Bael fruit. Fruit pulp will be collected. The pulp will be shade dried and powdered fine. The E. marmelos L. fruit pulp powder will be stored in airtight container. The powder will be used as per dosage with distilled water for rectal administration to the rats.
**Selection of material**
The plant material will be authenticated and identified from FRLHT Banglore or BSI Pune.

**Safety profile**
An acute oral toxicity study has been performed as per OECD-423 guidelines. Adult albino Sprague-Dawley rats were administered 5, 50, 300, and 2000 mg/kg body weight of aqueous extract of unripe fruit of A. marmelos orally. The control group received distilled water. The animals were observed for 14 days. There was no mortality or behavioural changes observed during the study period.

**Sampling Procedure & Sample Size**
24 Sprague-Dawley rats will be randomly divided into 4 groups. As per OECD guideline (Table 1).

**Table 1: Sample size:**
| Animals  | Sprague-Dawley rats |
|----------|---------------------|
| Sex      | 50% males and 50% females (nulliparous) in each group will be taken. |
| Weight (Average) | 200-250 gm. |
| Number of animals in each group | 06 rats |
| Number of groups | 04 |

**Induction of Osteoporosis and Intervention**
Osteoporosis (OP) will be induced in rats with steroid. All the rats in the disease control and experimental group will be given a steroid for 4 weeks and will be divided into the following groups (Table 2).

**Table 2: Groups and intervention:**

| Groups | Intervention | Drug | Dose | No. of animals and species | Duration | Route |
|--------|-------------|------|------|---------------------------|----------|-------|
| Group I (vehicle control group) | Distilled water | No drug | - | 6 rats | 12 wks | Oral |
| Group II (disease control group) | Distilled water | No drug | - | 6 rats | 12 wks | Oral |
| Group III (experimental group) | OP + Bael pulp | Unripe Bael fruit pulp powder with distilled water | 0.081 gm of powder /day (mean dose) | 6 rats | 12 wks | Oral |
| Group IV (experimental group) | OP + Bael pulp | Unripe Bael fruit pulp powder with distilled water | 0.060 gm of powder /day (mean dose) | 6 rats | 12 wks | Transrectal |

**Dose:**
The animal dose will be extrapolated from the human dose using the rat conversion factor.

Oral route: 3 gm - 6 gm of fruit pulp powder as per API. Rectal route: 75% (3/4) of the oral route

Total clinical dose (Human dose) x conversion factor (0.018) per 200 gm of rat Dose calculation for 4.5 gm of human dose (Mean dose)

For oral route: 4.5 x 0.018 = 0.081 gm of powder /day orally
For rectal route: 0.081 x 3/4 = 0.060 gm of powder/day rectally

**Duration of intervention:** The drug will be given for 12 weeks.

**Assessment parameters**

**Radiological parameters:**
Bone mineral density (BMD): The BMD of the femur will be evaluated

**Biochemical parameters:**
Osteocalcin (OCN), serum calcium, inorganic phosphate, TRAP 5b

**Histological study:**
The histological changes in the colon and periosteum will be studied with the help of expert histopathologists.

**Macroscopic study:**
After resection of the portion of colon, the mucosal surface will be graded with the help of a magnifying lens

**Microscopic study:**
A piece of colon will be fixed in phosphate-buffered formaldehyde, embedded in paraffin, sectioned (4 μm thick), and stained with hematoxylin and eosin. Each sample will be observed and evaluated under light microscope by two independent observers.
Variables:
Quantitative and qualitative variables will be used for the study. Quantitative variables will the values of biochemical parameters and bone mineral density whereas qualitative variables will be the histological study of the colon.

Analysis Plan: Student’s Paired t-test, one way ANOVA test.

RESULTS
Results will be assessed with the help of statistical test observations, biochemical parameters, bone mineral density, and histopathology reports.

CONCLUSION
At the end of the study, the conclusion will be made based on the results of Statistical Analysis biochemical parameters, bone mineral density, and histopathology reports. Osteoporosis is a metabolic disease. It affects a large population worldwide. Bone mass is reduced in osteoporosis and it leads to fractures of the bones making the people dependent on others. Current treatments like hormone replacement therapy, administration of calcium, cholecalciferol have its limitations and complications. Ayurvedic experts consider Purishdhara Kala is the Asthidhara Kala. It means that the Ayurvedic medicines which are effective in the diseases of the colon can be effective in the diseases of bones. To analyze the statement we have chosen the medicine, Bael (Aegle marmelos L.) that has well-known action on colon disorders. The Bael fruit is easily and abundantly available in India. It is cheaper also. With the proposed study we are looking forward to providing a reliable, safe and cheaper treatment for bone disorders. The study can provide a new aspect to the management of osteoporosis.

Limitations: Osteoporosis will be induced in animals artificially. There may be some kind of variation in the pathology of actual osteoporosis in human and osteoporosis due to induction. Nuances while handling animals in laboratory technique may cause alteration in the result. However, the disease arising due to pathological processor with the induction, manifestation of the disease remains the same. That’s why in the field of medical science, animal study has its importance. Further clinical studies with more precise biochemical parameters are needed to confirm the efficacy of the drug.

ACKNOWLEDGMENT
Authors acknowledge the immense help received from the scholars whose articles are cited and included in references to this manuscript. The authors are also grateful to authors / editors / publishers of all those articles, journals, and books from which the literature for this article has been reviewed and discussed.

Conflict of Interest: Nil
Source of Funding: Nil

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