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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 *PurishdharaKala* is the *AsthidharaKala* which is described in *visha-vegantara* in *kalpasthana*. This relationship has also clinical importance in Ayurvedic treatment. This relation has not yet been studied. As *Kala* is the structure intervening *Dhatu* and it's *Ashaya*, the *AsthiDhatu*(bones) is directly related to *AsthidharaKala*, 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 pre-clinical 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 "*Dhavantashayantar Maryada*" in classical Ayurvedic text. *Kalais* stratum or sheath existing between *Dhatu* and *Ashaya*. *Acharya Sushrut* has described *Kala Sharir* in *Sharirsthana* 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 *PurishdharaKala*.

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 *Kalain* 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."¹

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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.² 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.³

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 Kala* as 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.⁴ The common English names used to refer *A. Marmelos* are viz. wood apple, Bengal quince, golden apple, Indian quince, holy fruit, and stone apple.⁵ 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.⁶ Extract of Wood apple unripe fruit displays efficacy against inflammatory bowel disease (IBD) in Wistar albino rats.⁷ 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.⁸

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 Kal* 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 Kalait* 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 *Asthidhara Kala*.

2. To study the effect of administration of unripe *Bael* fruit (*Aegle marmelos* L.) on *Asthidhara Kalain* osteoporotic rats with oral and transrectal route
3. To study the effect of unripe *Bael* fruit (*Aegle marmelos* L.) on biochemical parameters selected 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 Rachana Sharir 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 ($22^{\circ}\text{C} \pm 2^{\circ}\text{C}$), the relative humidity of $55 \pm 5\%$ 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 air tight container. The powder will be used as per dosage with distil water for rectal administration to the rats.

Selection of material

The plant material will be authenticated and identified from FRLHT Bangalore 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.⁹

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 the 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 + <i>Bael</i> pulp	Unripe <i>Bael</i> fruit pulp powder with distilled water	0.081 gm of powder /day (mean dose)	6 rats	12 wks	Oral
Group IV (experimental group)	OP + <i>Bael</i> pulp	Unripe <i>Bael</i> 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.¹⁰

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: $4.5 \times 0.018 = 0.081$ gm of powder /day orally

For rectal route: $0.081 \times 3/4 = 0.060$ gm of powder/ day rectally

Duration of intervention: The drug will be given for 12weeks¹¹

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 be 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. Ayurvediya experts consider *Purishdhara Kalais 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.

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