Medicinal Plants for the Management of Post Menopausal Osteoporosis: A Review

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Abstract: Osteoporosis, a silent epidemic has become a major health hazard in recent years. Osteoporosis which increases bone fragility and thereby the risk of fractures is associated with high mortality, morbidity and high medical expenses throughout the world. Though ovarian hormone deficiency is a major risk factor for osteoporosis in the postmenopausal women, hormone replacement therapy (HRT), perhaps the most effective treatment, is not preferred as it increases the risk of breast cancer and of cardiovascular diseases. The other available therapeutic agents are also associated with certain adverse effects. In this context, phytoestrogens are believed to play a role in maintaining or improving skeletal health. The present work reviews scientific information on medicinal plants which have already been documented for their antiosteoporotic activity. These plants may differ from each other in their mechanisms of action, they either bind with estrogen receptors which exhibit responses at the cellular and molecular levels, or in some cases they act by improving defense against oxidative stress”. The review which covers 18 plants briefly discusses their morphology, family, common name, phytoconstituents and proposed mechanism of action.

Keywords: Antiosteoporotic, hormone replacement therapy (HRT), phytoestrogens, medicinal plants, postmenopausal osteoporosis.

INTRODUCTION

Osteoporosis, a silent epidemic has become a major health hazard in recent years, afflicting over 2000 million people worldwide [1]. It is a major growing health problem for elderly women associated with ovarian hormone deficiency following menopause and is by and far the most common cause of age related bone loss in women. According to the WHO “Osteoporosis is a disease characterized by low bone mass and microarchitectural deterioration of bone tissues, leading to enhanced fragility and consequent increase in fracture risk that results in fractures with minimal trauma”.

Osteoporosis is one of the most widespread metabolic bone disorders [2], affecting one in three women and one in twelve men at some point in their lives [3]. The risk of fracture has been reported to increase with age in humans [4]. A sharp decrease in ovarian estrogen production is the predominant cause of rapid, hormone-related bone loss during the first decade after menopause [5], as a result of higher bone turnover, an imbalance between bone formation and bone resorption & net bone loss [6].

The common sites of fracture among postmenopausal women include the vertebrae, forearm and hip. As the population ages, the incidence of hip fractures & cost for treatment will rise dramatically in the future, unless effective prophylactic measures are taken [7]. The projected cost of osteoporotic fractures in white postmenopausal women during the next 10 years in the United States alone is expected to be more than $45 billion [8]. Some epidemiological data suggests that in USA, 10 million individuals already have osteoporosis and 18 million have osteopenia making it to a total of 28 million, American women, however, are four times more likely to develop osteoporosis than men [9]. The incidence in Europe is projected to double in the next 50 years, and the incidence in Latin America is also expected to rise significantly [10]. Globally, osteoporosis is highest in Whites and Asians, and lowest among Blacks. Blacks have more bone density than other racial groups, lowering their risk of osteoporosis. Hispanic-American women have somewhat greater bone density than do non-Hispanic whites [11]. In India, based on 2001 census, approximately 163 million Indians are above the age of 50 and this number is expected to increase to 230 million by 2015 [12]. Even conservative estimates suggest that, of these, 20 per cent of women and about 10-15 per cent of men would be osteoporotic [13].

Several factors such as genetic, nutritional and lack of exercise etc., along with aging have been shown to be risk factors in the aetiology of osteoporosis [13]. With aging, however, an erratic absorption of calcium from gut disturbs the calcium homeostasis leading to an imbalance in the calcium regulating hormones (parathyroid hormone and calcitonin) and thereby increase bone turnover [14]. Osteoblastic activity and calcium absorption from the gut also suffers with the age [15]. In addition to menopause and aging, hereditary factors, lack of exercise or immobilization, lifestyle, prolonged steroid administration, excessive diet,
Methanol Extract of the Fruits of *Morinda citrifolia* Linn., Restores Bone Loss in Ovariectomized Rats

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3Department of QAU and Regulatory Affairs, Zydus Research Centre, Zydus Cadila, Ahmedabad, 382213, Gujarat, India

**Abstract:** The objective of this study was to evaluate the effect of methanol extract of the fruits of *Morinda citrifolia* Linn., on osteoporosis induced by ovariectomy in female albino rats at two different dose levels of 500 and 750 mg/kg/day. Healthy female albino rats in the age group of 90 days were selected and randomized into five groups of six animals each. Group 1 was sham operated and served as control while all the remaining groups were ovariectomized. Group 2 was fed with an equimol of saline and served as ovariectomized control. Group 3 was orally treated with standard Raloxifene (5.4 mg kg⁻¹) whereas the methanol extract of *Morinda citrifolia* (500 and 750 mg kg⁻¹) was administered to the groups 4 and 5. The findings assessed on the basis of biomechanical, biochemical and histopathological parameters, showed that the methanol extract significantly reduced bone loss, as evidenced by a reduction in Tartrate Resistant Acid Phosphatase (TRAP) and urine Hydroxyproline (Hp) levels while simultaneously increasing bone formation [high serum Alkaline Phosphatase (ALP) levels], thereby restoring bone mineralization. The restoration of bone strength was confirmed by biomechanical parameters viz., the three point bending of tibia, load testing of femoral head and compression of IV lumbar vertebra and it was further endorsed by histopathological findings i.e., bone microarchitecture. The extract significantly increased the osteoblastic activity on one hand while on the other it retarded the osteoclastic function thereby contributing to a positive bone balance and hence enhanced mineralization.

**Key words:**Osteoporosis, *Morinda citrifolia*, bone fragility, bone mineral density, tartrate resistant acid phosphatase (TRAP), alkaline phosphatase (ALP)

**INTRODUCTION**

Osteoporosis, a disease characterized by high bone fragility and increased risk of fractures with high mortality and morbidity rates, has become an expensive health menace worldwide (Meryl, 1997). Depletion of ovarian hormone following menopause is believed to be a major cause of brittle bones. Hormone Replacement Therapy (HRT), perhaps the most effective treatment, suffers the risk of breast cancer and cardiovascular diseases (Genant et al., 1989; Nand et al., 1999). A number of therapeutic agents based on various mechanisms of action, other than HRT have been developed for the management of osteoporosis e.g. antiresorptive agents [calcium and vitamin D (Jackson et al., 2006), bisphosphonates (Huq, 2007) including Selective Estrogen Receptor Modulators (SERMs) like Raloxifene (Delmas et al., 2005)] and bone forming agents [fluorides (Riggs et al., 1990, 2002) androgens (Christiansen and Ris, 1990), Parathyroid Hormone (PTH) (Lane et al., 1998) and phytoestrogens (Knight and Eden, 1996; Jaffery et al., 2006)]. The discovery of estrogenic activity of natural products (El-Halawany et al., 2010; Sreeja et al., 2010; Kim et al., 2010) and its scientific investigation have paved the way to a promising alternative mode of prophylaxis/treatment for osteoporosis. Many phytoconstituents have been designated as phytoestrogens and a number of plants containing these constituents have been identified and investigated in preclinical models for their estrogenicity (Cornwell et al., 2004; Hong et al., 2009).

*Morinda citrifolia* L. (Rubiacaeae), popularly known as Noni in India, is a reputed medicinal plant used to treat a wide variety of ailments in Polynesia, South east Asia, Australia and the Caribbean (Krishnaiah et al., 2009). 

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Methanol extract of dried exudate of *Commiphora mukul* prevents bone resorption in ovariectomized rats

Saleemulla Khan, Chandresh Dwivedi, Vinit Parmar, K. K. Srinivasan, and Annie Shirwaikar

**Department of Pharmacognosy, Manipal College of Pharmaceutical sciences, Manipal University, Madhavnagar, Manipal, India**

**Abstract**

**Context:** Gum guggul, a resinous exudate of the plant *Commiphora mukul* Engl. (Burseraceae), has been found efficacious in the treatment of bone fractures, arthritis, and hyperlipidemic disorders.

**Objective:** The present study is an effort to explore the anti-bone-resorptive potential of the dried methanol extract of the gummy exudate of *C. mukul* (MECM) in ovariectomized rat model.

**Materials and methods:** The animals were randomly divided into five groups of equal size (*n* = 6). Animals in all the groups were ovariectomized except group 1, which was sham operated. Groups 3, 4 and 5 were treated with Raloxifene, MECM 250 mg/kg and MECM 500 mg/kg, respectively. The 2nd group was fed with vehicle. Assessment: biochemical estimations, viz., alkaline phosphatase (ALP), tartrate resistant acid phosphatase (TRAP), serum calcium (Ca); biomechanical evaluations, and histopathological examinations.

**Results:** The LD<sub>50</sub> of MECM was found to be > 2500 mg/kg orally. A significant elevation was observed in the ALP, TRAP, Ca and cholesterol levels in the 2nd group with a significant reduction in biomechanical strength. Groups 3, 4 and 5, showed a significant reduction in TRAP and ALP levels (*p* < 0.001). The Ca levels were normalized in the groups 4 and 5, while cholesterol levels dropped in group 5. The bone strength, however, was normalized in all the groups (*p* < 0.001) along with the histopathology.

**Discussion and conclusion:** Findings suggested a significant gain in bone strength and nearly complete restoration of bone microarchitecture along with lowered levels of TRAP indicating the anti-bone resorptive potential of the extract.

**Keywords:** Osteoporosis, bone fragility, bone mineral density, biomechanical strength

**Introduction**

Osteoporosis is a disease characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to bone fragility and an increased susceptibility to fractures (Epstein, 2006). It results from a negative balance between the bone-forming activity of osteoblasts and the resorption activity of osteoclasts (Novack, 2007). It mainly affects post-menopausal women, elderly men and results in hip and vertebral fractures (Frost, 1992; Cooper et al., 1992). Hormone replacement therapy (HRT) is an established method in the prevention of fractures and bone loss in post-menopausal women; however, it is associated with an increased risk of breast cancer (Canfell et al., 2008), stroke and heart attacks (Botto et al., 2011), which are generally called estrogen-like side effects (Canderelli et al., 2007). Sodium alendronate, a bisphosphonate, is at present, one of the most popular medicines prescribed to treat osteoporosis. However, gastrointestinal intolerance to bisphosphonates has been reported in some patients (Szejnfeld, 2000). Osteonecrosis of the jaws (Somerman & McCauley, 2006) is another common adverse effect that has been associated with some of the bisphosphonates. Selective Estrogen Receptor Modulators (SERM), such as raloxifene, have also been reported to react adversely. Hot flushes and thromboembolic disorders are the most
Animal Ethical clearance

Department of Pharmacology
Kasturba Medical College
A Constituent College of Manipal Academy of Higher Education (A Deemed University)

Proceedings of the Institutional Animal Ethics Committee (IAEC)
meeting, KMC, Manipal

No. IAEC/KMC/96/2009-2010

Dated: December 26, 2009

To,
Mr. Saleemulla Khan
Sr. Lecturer
Dept. of Pharmacognosy
MCOPS, Manipal.

Through,
Dr.(Mrs.) Annie Shirwaikar
Professor
Dept. of Pharmacognosy
MCOPS, Manipal

Sir,

Subject: - Approval of your project entitled “Development of a herbal dietary supplement for the management of osteoporosis” by IAEC, MAHE.

The Institutional Animal Ethics Committee has scrutinised the above mentioned project and has approved it.

You are permitted to proceed with your project.

Dr. K. L. Bairy
Chairman
IAEC, MAHE
Manipal.
# Certificate of Analysis

<table>
<thead>
<tr>
<th><strong>Product Name</strong></th>
<th>β-Sitosterol, from soybean, ≥40%</th>
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<tr>
<td><strong>Product Number</strong></td>
<td>S5753</td>
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<tr>
<td><strong>Product Brand</strong></td>
<td>FLUKA</td>
</tr>
<tr>
<td><strong>CAS Number</strong></td>
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</tr>
<tr>
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<td>C\textsubscript{29}H\textsubscript{50}O</td>
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## TEST

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<tr>
<th><strong>Appearance (Color)</strong></th>
<th>White to Off-White</th>
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<tr>
<td><strong>Appearance (Form)</strong></td>
<td>Powder</td>
</tr>
<tr>
<td><strong>Solubility (Color)</strong></td>
<td>Light Yellow</td>
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<tr>
<td><strong>Solubility (Turbidity)</strong></td>
<td>Clear to Slightly Hazy</td>
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<td><strong>Content</strong></td>
<td>≥40 %</td>
</tr>
</tbody>
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50 mg/mL, CHCl\textsubscript{3}

B-Sitosterol (also contains campesterol and dihydrobrassicasterol as identified by C-13 NMR)

<table>
<thead>
<tr>
<th><strong>Proton NMR spectrum</strong></th>
<th>Conforms to Structure</th>
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</thead>
<tbody>
<tr>
<td><strong>13C NMR Spectrum</strong></td>
<td>Conforms to Structure</td>
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## SPECIFICATION

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<tr>
<td><strong>Appearance (Color)</strong></td>
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</tr>
<tr>
<td><strong>Appearance (Form)</strong></td>
<td>Powder</td>
</tr>
<tr>
<td><strong>Solubility (Color)</strong></td>
<td>Light Yellow</td>
</tr>
<tr>
<td><strong>Solubility (Turbidity)</strong></td>
<td>Very Slightly Hazy</td>
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<td><strong>Content</strong></td>
<td>44 %</td>
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Spec: SEP 2010

QC: SEP 2010

Print Date: SEP 09 2010

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Rodney Burbach, Manager
Quality Control
St. Louis, Missouri USA
Product Name:
(Z)-Guggulsterone – ≥89% (HPLC), powder

Product Number: GS168
Lot Number: 061M1144V
Brand: SIGMA
CAS Number: 39025-23-5
MDL Number: MFCD01310757
Formula: C21H28O2
Formula Weight: 312.45 g/mol
Storage Temperature: Store at 2-8 DEGREE C
Quality Release Date: 10 JUN 2011

<table>
<thead>
<tr>
<th>Test</th>
<th>Specification</th>
<th>Result</th>
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<tbody>
<tr>
<td>Appearance (Color)</td>
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<td>off-white</td>
</tr>
<tr>
<td>Appearance (Form)</td>
<td>Powder</td>
<td>Powder</td>
</tr>
<tr>
<td>Solubility</td>
<td>Conforms</td>
<td>Conforms</td>
</tr>
<tr>
<td>Soluble at 5 mg/mL, DMSO</td>
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<td></td>
</tr>
<tr>
<td>Carbon</td>
<td>79.0 - 82.3 %</td>
<td>80.7 %</td>
</tr>
<tr>
<td>Proton NMR spectrum</td>
<td>Conforms to Structure</td>
<td>Conforms</td>
</tr>
<tr>
<td>13C NMR Spectrum</td>
<td>Conforms to Structure</td>
<td>Conforms</td>
</tr>
<tr>
<td>Purity (HPLC)</td>
<td>≥ 89 %A</td>
<td>99 %A</td>
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<tr>
<td>Z-Isomer</td>
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<tr>
<td>% (E)-isomer (%A by HPLC)</td>
<td>≤ 5.4</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Rodney Burbach, Manager
Analytical Services
St. Louis, Missouri US

Sigma-Aldrich warrants, that at the time of the quality release or subsequent retest date this product conformed to the information contained in this publication. The current Specification sheet may be available at Sigma-Aldrich.com. For further inquiries, please contact Technical Service. Purchaser must determine the suitability of the product for its particular use. See reverse side of invoice or packing slip for additional terms and conditions of sale.
Certificate of Analysis

Product Name: Lupeol, ≥94%
Product Number: L5632
Product Brand: ALDRICH
CAS Number: 545-47-1
Molecular Formula: C_{30}H_{50}O
Molecular Weight: 426.72

TEST
APPEARANCE: WHITE POWDER
SOLUBILITY: CLEAR COLORLESS SOLUTION
SPECIFIC ROTATION: +26.9 DEG (C = 4.8 IN CHLOROFORM AT 20 DEG CELSIUS)
PURITY BY THIN LAYER CHROMATOGRAPHY: 98%
QC RELEASE DATE: MARCH 2008

Rodney Burbach, Manager Quality Control
St. Louis, Missouri USA
Addendum to Chapter 7.

7.3.3.2.3 Real time stability studies using stability chambers (Electrolab Mumbai)

Table 7-17 Physical characteristics of tablets after one year.

<table>
<thead>
<tr>
<th>Time</th>
<th>Color and shape</th>
<th>Thickness</th>
<th>Friability</th>
<th>Weight</th>
<th>Hardness (kg/cm²)</th>
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</thead>
<tbody>
<tr>
<td>Oth day</td>
<td>Dark brown</td>
<td>0.74±0.021</td>
<td>0.44±0.054</td>
<td>721 mg</td>
<td>5.5 ± 0.02</td>
</tr>
<tr>
<td>After 3 months</td>
<td>Dark brown</td>
<td>0.74±0.016</td>
<td>0.44±0.126</td>
<td>721 mg</td>
<td>5.4 ± 0.06</td>
</tr>
<tr>
<td>After 6 months</td>
<td>Dark brown</td>
<td>0.74±0.024</td>
<td>0.44±0.16</td>
<td>721 mg</td>
<td>5.2 ± 0.102</td>
</tr>
<tr>
<td>After 1 year</td>
<td>Dark brown</td>
<td>0.74±0.011</td>
<td>0.47±0.086</td>
<td>721 mg</td>
<td>5.2 ± 0.04</td>
</tr>
</tbody>
</table>

Photographs of Tablets along with packing material for stability studies.

Fig: A1; Tablets with packing material for stability studies. T. Tablet, S. Screw Cap, C. Cotton, B. HDPE bottle

Fig: A2; Tablets and HDPE bottle for packing tablets for stability studies