Anti-osteoporotic activity of methanolic extract of an Indian herbal formula NR/ICAL/06 in ovariectomized rats

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Objective: The present study was aimed to evaluate the anti-osteoporotic activity of methanolic extract of NR/ICAL/06 in ovariectomized (OVX) rats.

Methods: Bilateral ovariectomy was performed in female Sprague-Dawley rats under aseptic conditions and the rats were divided into five groups (n=10). Two different doses of methanolic extract of NR/ICAL/06 (200 and 400 mg/kg, p.o.) were evaluated for anti-osteoporotic activity and raloxifene (5.4 mg/kg, p.o.) was used as a reference standard. Treatment was given for 90 d. Anti-osteoporotic potential of NR/ICAL/06 was evaluated based on various parameters, namely, body weight, organ weight, bone weight, bone mineral content, bone strength, calcium and phosphorus in serum and urine, and serum alkaline phosphatase (ALP). External diameter, length and thickness of the femur bone were measured by using scanning electron microscopy (SEM).

Results: The bilateral ovariectomy in rats resulted in decreased bone strength, bone mineral content and bone weight. The SEM images showed porous, perforated and disintegrated femur bone architecture and decreases in bone length and thickness in OVX rats. These changes were associated with elevated serum levels of calcium, phosphorus and ALP. Increases in body weight and adipose weight and a decrease in uterine weight were also observed and the changes were highly significant when compared with the sham-control group. Treatment with methanolic extract of NR/ICAL/06 (200 and 400 mg/kg, p.o.) for 90 d dose-dependently restored the ovariectomy-induced alterations in bone weight, bone mineral content, bone strength, serum calcium, phosphorus and ALP, body weight and adipose weight nearly to normal levels. Furthermore, the SEM images of the femur bones of NR/ICAL/06 (200 and 400 mg/kg, p.o.)-treated rats showed reduced pore formation and improved bone compactness compared with the OVX-control group.

Conclusion: These findings suggest that the methanolic extract of NR/ICAL/06 possesses significant anti-osteoporotic activity and may be useful in the treatment of osteoporosis.

Keywords: osteoporosis; ovariectomy; plant extracts; medicine, Ayurvedic; microscopy, electron; scanning; rats

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Osteoporosis is a disease of aging associated with bone loss, and often occurs without symptoms, until the bone fracture occurs due to significant microarchitectural deterioration not possible to detect, hence it is of great importance in health care. Although reduced in mass, the bones are normal with respect to mineralization; however, histologically there could be significant decreases in the thickness of the cortex and the number and size of trabeculae of the coarse cancellous bone[1-3].

Phytoestrogens are the plant-derived substances; they are structurally and functionally similar to estrogens and are found in many foods. They exhibit estrogenic activity by acting on estrogen receptors. They have both weak estrogen and antiestrogenic activity; hence they are termed as natural selective estrogen receptor modulators (SERMs). Their SERM-like action makes them very useful in various indications[4].

Modern therapy recommended for the treatment of osteoporosis includes supplementation with estrogen, progesterin, calcitonin and bisphosphonates[5, 1]. Estrogen replacement therapy is the most widely recommended method to reduce the rate of postmenopausal bone loss, because estrogen is the most potent inhibitor of bone resorption[6, 7]. However, this therapeutic management possesses several downsides and available evidence suggests that long-term use of estrogen replacement therapy may have serious side effects such as breast or/ and uterus cancer[8]. Currently SERMs such as raloxifene and various types of bisphosphonates are used for beneficial effects on bone mineral density[9]; however, serious side effects, such as hypercalcemia, hypercalciuria, increased risk of endometrial and breast cancer, breast tenderness, irregular menstruation, thromboembolic events, vaginal bleeding and hot flushes were also reported[10]. Scientists throughout the world are looking for better alternative therapeutic management especially from natural resource.

In the traditional system of Ayurvedic medicine, plant species such as Glycyrrhiza glabra, Cestrum diurnum and Hibiscus rosasinensis have been reported for calcium enhancement and estrogen-like activity. The NR/CAL/06 is an herbal formulation consists of mixture of three different plant parts namely, Hibiscus rosasinensis (flowers), Cestrum diurnum (leaves) and Glycyrrhiza glabra (whole plant) in the ratio of 1:1:1:

Hibiscus rosasinensis Linn. is a native of China and its flowers have been reported to possess anti-implantation and antispermatogenic activities and it is also been scientifically proved for anti-implantation and uterotrophic activities[11].

Cestrum diurnum, is a single or multistemmed shrub or rarely a small tree that is also known as day cestrum, wild jasmine, inkbush and Chinese inkberry (English). The leaves of the plant contain a calcinogenic glycoside called 1,25-dihydroxycholecalciferol that helps in synthesis of vitamin D, hence it is thought to be useful in the treatment of osteoporosis[12].

Glycyrrhiza glabra is found to contain triterpene saponins, flavonoids, polysaccharides, pectins, simple sugars, amino acids, mineral salts and various other substances[13]. The isoflavones, glabridin, hispaglabridin A and hispaglabridin B have significant antioxidant activity and both glabridin and glabrene possess estrogen-like activity[14, 15].

Based on the literature reports, the three plant parts were selected and the combination of these plant parts (NR/CAL/06) were thought to be very useful in the management of osteoporosis and hence the present study was undertaken to evaluate the anti-osteoporotic activity of methanolic extract of NR/CAL/06.

1 Materials and methods

1.1 Chemicals Raloxifene (Dr. Reddy’s Laboratories Ltd., Hyderabad, India), ketamine (Neon Laboratories Ltd., Thane, India), xylazine (Xylazine, Indian Immunologicals Ltd., Andhra Pradesh, India).

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Pradesh, India), and biochemical and electrolyte estimation kits (Span Diagnostic Ltd., Surat, India) were used; all other solvents and chemicals used for the study were of analytical grade and purchased from local firms.

1.2 Extraction procedure The herbal formulation NR/CAL/06 was gifted and authenticated by M/s Natural Remedies Pvt. Ltd., Bangalore, Karnataka, India. Air dried parts of the plants were used for the formulation and the formulation was successively extracted in Soxhlet apparatus using methanol (70%) at 60 to 80°C. The extract was then concentrated by distilling the solvent, evaporating them to dryness at low temperature and the percentage extractive values were calculated in terms of air dried weight of the plant material.

1.3 Experimental animals Female Sprague-Dawley rats (150 to 200 g, 8 to 10 weeks old) were purchased from Vivo Bioscience, Bangalore, Karnataka, India. All the animals were acclimatized for 7 d under standard husbandry conditions, that is, room temperature of (25 ± 1) °C, relative humidity 45% to 55% and a 12:12 h light/dark cycle. The rats had free access to standard rat pellet with water supplied ad libitum. The approval of the Institutional Animal Ethical Committee (IAEC) of P.E.S College of Pharmacy, Bangalore, Karnataka, India, was taken prior to the experiments and all the experiments were conducted in strict compliance according to the ethical principles and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

1.4 Acute toxicity Acute oral toxicity of methanolic extract of NR/CAL/06 was determined by using female, nulliparous and non-pregnant mice weighing 18 to 22 g. The mice were fasted for 3 h prior to the experiment. Up and down procedure OECD (Organisation for Economic Co-operation and Development) guideline No. 425 was adopted for toxicity studies (http://www.epa.gov/oppeadl/harmonization/). Mice were administered with a single dose of extract and observed for their mortality during 48 h (acute) and 14 d (chronic). Lethal dose 50 (LD₅₀) was calculated as per OECD No. 425 using acute oral toxicity (AOT) 425 stat program.

1.5 Experimental procedure After one week of acclimatization, the rats were anaesthetized with ketamine plus xylazine ((75+5) mg/kg, intraperitoneally (i.p.)) and bilateral ovariectomy was performed under aseptic conditions. Similarly, sham operation was performed by only exposing the ovaries. All the operated rats were prophylactically treated with amoxicillin (25 mg/kg, i.p.) and povidone-iodine solution was applied locally for 4 d.

After complete surgical recovery, all the operated rats were randomly divided into five groups (G1-G5) of 10 animals in each. G1 was sham-operated and served as a basal control. All the other groups were ovarioctomized (OVX) and received treatment for 90 d, starting from the 15th day of ovarioctomy. G2 was vehicle control and served as OVX control; G3 was standard control given standard drug raloxifene (5.4 mg/kg, per oral (p.o.)); G4 and G5 were orally treated with suspension of methanolic extract of NR/CAL/06 at 200 and 400 mg/kg, p.o., in 0.5% carboxy methyl cellulose (CMC), respectively for 90 d. The body weights were recorded on day 0 and subsequently on every week till the end of the experiment and the body weight changes were derived by using days 0, 45 and 90 body weights. At the end of the treatment, blood and urine samples (using metabolic cages) were collected from all the animals to assess the biochemical parameters and they were sacrificed by over-dose of ether anesthesia; both the femur bones were isolated; femur weight, length and thickness were measured and recorded. Simultaneously uterus and adipose tissues were also removed and weighed. Further, the femur bones were used for the estimation of bone mineral content, biomechanical testing and scanning electron microscopy (SEM).

1.6 Measuring indexes for anti-osteoporotic activity

1.6.1 Biochemical parameters The calcium (serum and urine), phosphorus (serum and urine) and serum alkaline phosphatase (ALP) were estimated by using diagnostic reagent kits (Span Diagnostic Ltd., Surat, India) and according to the procedures given in respective kit inserts.

1.6.2 Bone mineral content Bone (left femur) ashes were prepared in a muffle furnace (700°C for 6 h) and dissolved in 0.1 mol/L hydrochloric acid solution. Bone minerals (calcium and phosphorus) were measured by a UV-visible spectrophotometer (RMS-BCA 201).

1.6.3 Three-point bending of femur The isolated bones (right and left femur) were assessed for their biomechanical strength by using the tensile strength testing machine. The sample was mounted horizontally between the mounting slots of the apparatus (Zwick/Roell 2005) against the cylindrical stoppers of 5 mm diameter; the cylindrical stoppers supporting the bone at ends are rigidly fixed to the frame of the apparatus. Load was applied exactly at the center of the sample between the end supports by means of a steel wire of 0.5 mm diameter passing horizontally. Load was varied gradually from 5 N to maximum breaking point value for the sample by increments of small steps and corresponding deflection of the sample was assessed by means of a laser displacement sensor which is set to measure the deflection of the sample at the loading point. The displacement sensor was connected to a computer through a data acquisition system which continuously acquired and stored the displacement data in the computer.
1.6.4 SEM evaluation External diameter, length and thickness of femur bones were measured with the help of a digital calliper (Mitutoyo, Japan). For SEM, the femur bones were fixed with phosphate formalin buffer, then dehydrated with graded concentrations of ethanol and coated with gold. The processed bones were then analyzed at 20 kV accelerating voltage by a SEM (JEOL, JSM-840A, Japan).

1.7 Statistical analysis All the values were expressed in terms of mean ± standard error of mean at each dose level. The level of significance was determined by one-way analysis of variance followed by Tukey’s post-hoc test. *P* < 0.05 was considered significant.

2 Results

2.1 Acute toxicity In acute toxicity study, the methanolic extract of NR/CAL/06 did not produce any mortality or toxic signs till 2000 mg/kg, p.o. Hence, the methanolic extract of NR/CAL/06 was found to be safe till 2000 mg/kg, p.o.

2.2 Anti-osteoporotic activity

2.2.1 Body weight The overall body weight analysis at the end of the study revealed that the OVX-control group showed a significant weight gain compared with the sham-control group (*P* < 0.01). The raloxifene (5.4 mg/kg) and NR/CAL/06 (200 and 400 mg/kg) inhibited the weight gain and maintained the body weight changes near to the body weight range of the sham-control group (*P* < 0.01) (Table 1).

2.2.2 Adipose mass, uterine weight and weight, length and thickness of femur The OVX-control group rats showed a decrease in uterine weight (*P* < 0.01) and femur weight (*P* < 0.05) and thickness (*P* < 0.05) when compared with the sham-control group, whereas the adipose mass in the OVX-control group was significantly more than that of the sham-control group (*P* < 0.01). The raloxifene and NR/CAL/06 (200 and 400 mg/kg) treatments significantly restored the ovariectomy-induced changes in adipose mass (*P* < 0.01, *P* < 0.05), uterine weight (*P* < 0.01) and femur weight (*P* < 0.01) and thickness (*P* < 0.05) compared with the OVX-control group. However, there was no change in the femur length in any of the groups (Table 2).

2.2.3 Serum biochemical constituents OVX-control rats showed a significant rise in serum ALP levels (*P* < 0.01) and there was gradual rise in serum calcium (*P* < 0.01) and phosphorus levels (*P* < 0.01) compared with the sham-control rats. Raloxifene (5.4 mg/kg, p.o.) and NR/CAL/06 (200 and 400 mg/kg) treatments significantly normalized the elevated levels of serum ALP (*P* < 0.01), calcium (*P* < 0.01) and phosphorus (*P* < 0.01) when compared with the OVX-control group (Table 3).

2.2.4 Urine biochemical constituents and bone mineral content The urine samples of the OVX-control rats collected on the 90th day showed a significant increase in urine calcium (*P* < 0.01) and phosphorus levels (*P* < 0.05) compared with the sham-control group; corresponding ash weight (*P* < 0.01) and ash calcium (*P* < 0.01) were significantly decreased, but there was no significant change in ash phosphorus content in the OVX-control group compared with the sham-control group. These changes were significantly normalized in rats treated with raloxifene (5.4 mg/kg, p.o.) and NR/CAL/06 (200 and 400 mg/kg, p.o.) (Table 4).

### Table 1 Effects of methanolic extract of NR/CAL/06 on body weight of rats with ovariectomy-induced osteoporosis

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Body weight (g)</th>
<th>Increase in body weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 0</td>
<td>Day 45</td>
</tr>
<tr>
<td>Sham-control</td>
<td>10</td>
<td>198.20±3.43</td>
<td>209.10±6.87</td>
</tr>
<tr>
<td>OVX-control</td>
<td>10</td>
<td>213.10±9.26</td>
<td>233.70±8.18</td>
</tr>
<tr>
<td>Raloxifene (5.4 mg/kg)</td>
<td>10</td>
<td>209.60±13.19</td>
<td>222.29±14.42</td>
</tr>
<tr>
<td>NR/CAL/06 (200 mg/kg)</td>
<td>10</td>
<td>216.87±38.63</td>
<td>229.90±41.90</td>
</tr>
<tr>
<td>NR/CAL/06 (400 mg/kg)</td>
<td>10</td>
<td>208.80±5.38</td>
<td>226.45±4.77</td>
</tr>
</tbody>
</table>

* **P* < 0.01, vs sham-control group; ΔΔ* P* < 0.01, vs OVX-control group. OVX: ovariectomized.

### Table 2 Effects of methanolic extract of NR/CAL/06 on adipose mass, uterine weight and weight, length and thickness of femur in rats with ovariectomy-induced osteoporosis

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Adipose mass (g)</th>
<th>Uterine weight (g)</th>
<th>Femur weight (g)</th>
<th>Femur length (mm)</th>
<th>Femur thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-control</td>
<td>10</td>
<td>2.642±0.775</td>
<td>0.383±0.014</td>
<td>0.713±0.021</td>
<td>35.028±0.760</td>
<td>3.907±0.120</td>
</tr>
<tr>
<td>OVX-control</td>
<td>10</td>
<td>3.857±0.747**</td>
<td>0.173±0.043**</td>
<td>0.690±0.178*</td>
<td>35.957±2.007</td>
<td>3.792±0.949*</td>
</tr>
<tr>
<td>Raloxifene (5.4 mg/kg)</td>
<td>10</td>
<td>1.907±0.394ΔΔ</td>
<td>0.223±0.035ΔΔ</td>
<td>0.808±0.166ΔΔ</td>
<td>35.853±7.310</td>
<td>3.905±0.797Δ</td>
</tr>
<tr>
<td>NR/CAL/06 (200 mg/kg)</td>
<td>10</td>
<td>3.107±0.608ΔΔ</td>
<td>0.220±0.051ΔΔ</td>
<td>0.810±0.168ΔΔ</td>
<td>35.797±2.926</td>
<td>3.862±0.753Δ</td>
</tr>
<tr>
<td>NR/CAL/06 (400 mg/kg)</td>
<td>10</td>
<td>2.703±0.394ΔΔ</td>
<td>0.297±0.095ΔΔ</td>
<td>0.818±0.170ΔΔ</td>
<td>36.210±7.562</td>
<td>3.933±0.823Δ</td>
</tr>
</tbody>
</table>

* *P* < 0.05, ** *P* < 0.01, vs sham-control group; ΔΔ* P* < 0.05, ΔΔ* P* < 0.01, vs OVX-control group. OVX: ovariectomized.
### Table 3 Effects of methanolic extract of NR/CAL/06 on serum biochemical constituents of rats with ovariectomy-induced osteoporosis

<table>
<thead>
<tr>
<th>Group</th>
<th>Calcium (mg/dL)</th>
<th>Phosphorus (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 45</td>
</tr>
<tr>
<td>Sham-control</td>
<td>10</td>
<td>10.08±0.19</td>
</tr>
<tr>
<td>O VX-control</td>
<td>10</td>
<td>10.09±0.17</td>
</tr>
<tr>
<td>Raloxifene (5.4 mg/kg)</td>
<td>10</td>
<td>9.98±0.11</td>
</tr>
<tr>
<td>NR/CAL/06 (200 mg/kg)</td>
<td>10</td>
<td>10.16±0.14</td>
</tr>
<tr>
<td>NR/CAL/06 (400 mg/kg)</td>
<td>10</td>
<td>10.29±0.13</td>
</tr>
</tbody>
</table>

*P<0.01, vs sham-control group; △ P<0.05, △△ P<0.01, vs O VX-control group. O VX: ovariectomized; ALP: alkaline phosphatase.

### Table 4 Effects of methanolic extract of NR/CAL/06 on urinary biochemical constituents and ash parameters in rats with ovariectomy-induced osteoporosis

<table>
<thead>
<tr>
<th>Group</th>
<th>Urine calcium (mg/dL)</th>
<th>Urine phosphorus (mg/dL)</th>
<th>Urine ash weight (g)</th>
<th>Ash calcium (mg/g Ash)</th>
<th>Ash phosphorus (mg/g Ash)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 45</td>
<td>Day 90</td>
<td>Day 0</td>
<td>Day 45</td>
</tr>
<tr>
<td>Sham-control</td>
<td>10</td>
<td>4.860±0.534</td>
<td>9.860±0.634</td>
<td>0.650±0.034</td>
<td>11.6±0.12</td>
</tr>
<tr>
<td>O VX-control</td>
<td>10</td>
<td>8.190±0.374**</td>
<td>11.200±0.774*</td>
<td>0.380±0.054**</td>
<td>10.6±0.44**</td>
</tr>
<tr>
<td>Raloxifene (5.4 mg/kg)</td>
<td>10</td>
<td>4.930±0.688△△</td>
<td>7.840±0.688△△</td>
<td>0.670±0.059△△</td>
<td>10.8±0.13△△</td>
</tr>
<tr>
<td>NR/CAL/06 (200 mg/kg)</td>
<td>10</td>
<td>6.350±0.513△△</td>
<td>8.750±0.813</td>
<td>0.600±0.055</td>
<td>11.3±0.11△△</td>
</tr>
<tr>
<td>NR/CAL/06 (400 mg/kg)</td>
<td>10</td>
<td>6.160±0.374△△</td>
<td>8.610±0.674</td>
<td>0.620±0.053△△</td>
<td>11.9±0.10△△</td>
</tr>
</tbody>
</table>

*P<0.05, ** P<0.01, vs sham-control group; △ P<0.05, △△ P<0.01, vs O VX-control group. O VX: ovariectomized.

### 2.3 Three-point bending of femur

The O VX-control rats showed a significant decrease in bone strength due to deficiency of estrogen compared with the sham-control group (P<0.01). Treatments of raloxifene (5.4 mg/kg) (P<0.01) and NR/CAL/06 (400 mg/kg, p.o.) (P<0.01) significantly restored the ovariectomy-induced altered bone strength. However, raloxifene (5.4 mg/kg) was more potent than NR/CAL/06 (400 mg/kg). NR/CAL/06 (200 mg/kg, p.o.) did not offer any significant restoration of ovariectomy-induced altered bone strength (Table 5).

### Table 5 Effects of NR/CAL/06 on bone mechanical strength determined by 3-point bending in ovariectomized rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Force (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-control</td>
<td>10</td>
</tr>
<tr>
<td>O VX-control</td>
<td>10</td>
</tr>
<tr>
<td>Raloxifene (5.4 mg/kg)</td>
<td>10</td>
</tr>
<tr>
<td>NR/CAL/06 (200 mg/kg)</td>
<td>10</td>
</tr>
<tr>
<td>NR/CAL/06 (400 mg/kg)</td>
<td>10</td>
</tr>
</tbody>
</table>

** P<0.01, vs sham-control group; △△ P<0.01, vs O VX-control group. O VX: ovariectomized.

### 2.4 SEM evaluation

The SEM images of O VX-control rats showed alterations of normal bone architecture and morphology such as pore formation, disintegrated bone architecture, reduced compactness. Treatment with raloxifene and NR/CAL/06 (200 and 400 mg/kg) restored the ovariectomy-induced morphological abnormalities (reduced pore formation, calcified cartilaginous deposits, improved bone architecture and increased compactness) to normal levels (Figure 1).

### 3 Discussion

The present study was undertaken to evaluate the anti-osteoporotic activity of methanolic extract of NR/CAL/06, an herbal formulation, in O VX rats. It is well known that estrogen deficiency is one of the important risk factors in the pathogenesis of osteoporosis. Bilateral ovariectomy results in dramatic decreases in uterine weight, bone mineral density and biomechanical strength due to deficiency of estrogen.

Scientifically it has been proved that, human diet contains phytoestrogens which can be useful in the prevention and treatment of osteoporosis[18]. Soybean isoflavones have structures similar to those of the estrogen and have received attention as alternatives to hormone replacement therapy for the prevention of postmenopausal osteoporosis. Thus, phytoestrogens are said to be protective against osteoporosis due to their ability to exert oestrogenic actions on bone cells in postmenopausal women and thus suppressing osteoclastic bone resorption and promoting bone formation[117, 18].

In the present study, oral administration of methanolic extract of NR/CAL/06 for 90 d significantly restored the ovariectomy-induced abnormalities in bone biomechanical strength, bone mineral content and uterine weights to normal levels; similarly the reference drug raloxifene (5.4 mg/kg, p.o.) is a SERM, has also restored the ovariectomy-induced abnormalities to normal levels. These results suggest that NR/CAL/06 may act as an estrogen agonist, as it has offered similar effect as estrogen on bone loss and uterine weight.

In the present study, the body weight changes and serum ALP levels support the observations of the other investigators related to increased body weight and elevated serum ALP levels due to ovarian hormone deficiency[10]. The administration of NR/CAL/06 (200 and 400 mg/kg) or raloxifene (5.4 mg/kg) significantly normalized the ovariectomy-induced increases in body weight and elevated serum ALP.

Calcium and phosphorus are widely accepted phenotype markers for bone formation[10]. In the present study, treatment with NR/CAL/06 or raloxifene restored the decreased serum calcium and phosphorus concentrations to normal levels. Our findings showed that, the bilateral ovariectomy developed the bone changes similar to those seen in the estrogen-deficient osteoporotic women, most markedly is decrease in bone density. Administration of NR/CAL/06 (200 and 400 mg/kg) or raloxifene (5.4 mg/kg) significantly improved the bone density and therefore effective in preventing the bone loss due to estrogen deficiency. Bone strength is related to bone density, architecture, connectivity and mineralization[21]. Biomechanical testing indicated that the OVX-control rats have low mechanical strength compared with the sham-control rats. Treatment with NR/CAL/06 (200 and 400 mg/kg) or raloxifene (5.4 mg/kg) greatly strengthened the biomechanical properties in OVX rats.

In addition, the SEM images of the rats treated with NR/CAL/06 (200 and 400 mg/kg) and raloxifene (5.4 mg/kg) showed improved ossification, bone architecture, mineralization and calcified cartilaginous deposits compared with the OVX-control rats.

4 Conclusion
In conclusion, these findings demonstrated the beneficial effects of methanolic extract of NR/CAL/06 when orally administered to rats with ovariectomy-induced osteoporosis. The methanolic extract of NR/CAL/06 could be considered as a natural alternative to hormone replacement therapy for the prevention and treatment of bone loss in postmenopausal women. Further studies will be needed to determine whether vitamin D and phytoestrogen derivatives might have direct actions on bones as well as to identify other bioactive compounds that might account for its bone-protective effects in vivo.

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6 Conflicts of interest
Authors declare that there is no conflict of interest.
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印度草药方 NR/CAL/06 的甲醇提取物
对卵巢切除大鼠的抗骨质疏松作用

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目的：证实印度草药方 NR/CAL/06 的甲醇提取物对卵巢切除大鼠的抗骨质疏松作用。

方法：雄性 Sprague-Dawley 大鼠在无菌状态下行双侧卵巢切除术后被分为 5 组 (n = 10)。检测 NR/CAL/06 的甲醇提取物 (200 和 400 mg/kg 口服) 的抗骨质疏松作用并以氟洛普芬 (5.4 mg/kg 口服) 作为标准对照药，治疗时间为 90 d。实验前后测量各组大鼠的体质质量、卵巢质量、骨质量、骨矿物质含量、骨强度、血清及尿液中的钙、磷酸含量及血清碱性磷酸酶含量。电镜下观察大鼠股骨外径、长度及厚度。

结果：双侧卵巢切除术后，大鼠骨质量、骨矿物质含量及骨强度均有下降；电镜观察到卵巢切除大鼠股骨质疏松、多孔、破碎，且骨长度及厚度均有所下降。与假手术组相比，卵巢切除大鼠的血清钙、磷及碱性磷酸酶含量均有所升高，体质量及脂肪含量升高，子宫质量降低。NR/CAL/06 的甲醇提取物 (200 和 400 mg/kg 口服) 治疗 90 d 能够剂量依赖性地改变大鼠因卵巢切除所引起的上述各项指标变化，并将骨质量、骨矿物质含量、骨强度、血清钙、磷及碱性磷酸酶含量，体质量及脂肪含量恢复至正常水平。此外，经 NR/CAL/06 的甲醇提取物 (200 和 400 mg/kg 口服) 治疗的大鼠与卵巢切除模型组大鼠相比，电镜下股骨骨质孔形成减少，骨密度增加。

结论：本实验的结果证实了 NR/CAL/06 的甲醇提取物具有很好的抗骨质疏松作用，可用于骨质疏松症的治疗。

关键词：骨质疏松；卵巢切除术；植物提取物；医学；印度传统；显微镜检查；电子；扫描；大鼠