Effect of calcarea carbonica on osteoporotic activities in ovariectomised albino rat model of osteoporosis in 1x, 30, and 0/1 potencies

Winston Vargheese¹, Alphy Mathew¹*, C.K. Mohan² and Krishna Kumar Amma. C.R¹

¹Department of of Materia Medica, Sarada Krishna Homoeopathic Medical College, Kulasekharam, TN, India
²Department of Paediatrics, Sarada Krishna Homoeopathic Medical College, Kulasekharam, TN, India
*Corresponding Author E-mail: alphym2@gmail.com (Mathew)

ABSTRACT

Objective: To study the effect of Calcarea Carb, in osteoporotic activity, in ovariectomized rat model of osteoporosis with 3 different potency scales -1X, 30 and 0/1, to compare the effect of Calcarea Carb with estrogen in controlling osteoporosis and to study the potency that is more suitable in treating osteoporosis, in ovariectomized rat model of osteoporosis.

Materials and Methods: Healthy female Wistar albino rats were divided into 5 groups of 6 animals each. Ovariectomy was done for all the groups. Group 1(control) was fed with saline. Group 2 was orally treated with estrogen (2mg/kg) and Groups 3-5 were orally treated with Calcarea Carb in potencies 1X, 30 and 0/1 respectively, for 90 days. After 90 days the rats were sacrificed for bone physical parameters, uterine weight, body weight, estimation of serum levels of calcium, phosphorous, alkaline phosphatase and osteocalcin and also for histopathological estimation.

Result: For all parameters, except body weight and histopathological examination, Calcarea Carb 1X showed higher result. For the histopathological estimation of femur and body weight, Calcarea Carb 0/1 showed the highest result.

Conclusion: Calcarea Carb is effective in controlling osteoporosis in all the potencies, particularly in 0/1 potency.

Keywords: Osteoporosis, Calcarea Carb, Ovariectomy, Osteocalcin, Alkaline phosphatase


INTRODUCTION:

Osteoporosis is a skeletal disorder, characterized by a loss of bone osteoid, that reduces the bone integrity and bone strength, leading to an increased risk of fractures [1,2]. Loss of bone tissue is associated with deterioration in skeletal micro-architecture. The WHO operationally defines osteoporosis as, a bone density that falls 2.5 standard deviations (SD) below the mean for young healthy adults of the same gender-also referred to as a T-score of -2.5. Post-menopausal women who fall at the lower end of the young normal range (a T-score of >1 SD below the mean) are defined as having low bone density and are also at increased risk of osteoporosis [1]. It is the most common cause for broken bone among the elderly. Patients with osteoporosis are asymptomatic, until a fracture occurs. Osteoporosis is the second most common metabolic bone disease in India [3]. Presently it is estimated that more than 200 million people worldwide suffer from osteoporosis. 30% of all post-menopausal women, on an average, have osteoporosis in U.S and Europe. At least 40% of these women and 15-30% of men will sustain one or more fragility fracture [4]. Osteoporosis is responsible for more than 1.5 million fractures annually [5]. Calcium is an important component of the skeletal tissue which contributes to its strength. In post-menopausal age groups, with loss of estrogen, there will also be an alteration in the calcium metabolism, resulting in low calcium levels. This can result in reduction of the bone mineral density, thus contributing to fractures. Homoeopathic remedy, Calcarea Carb, which is nothing but calcium carbonate, prepared from the
middle layer of oyster shell. Calcarea Carb has marked action on the bones [6]. Calcarea Carb makes changes in the composition of blood, by modifying the nutrition of the vegetative system, which finally terminates in certain constitutional diseases [7]. Calcium and phosphate ions precipitate as apatite. Apatite is an insoluble substance that helps in the calcification of cartilage in bone formation. During acidic conditions, calcium ions get immobilized from calcium deposits. This can lead to osteoporosis [8]. From a retrospective study conducted on Calcarea Carb, it was found that Calcarea Carb has a great affinity for musculoskeletal system and also for females between age groups 36-60 years, which mainly includes menopausal women. Menopause can result in osteoporotic changes [9]. In the modern era of evidence-based medicine, we wish to prove the same experimentally with ovariectomized albino rats.

MATERIALS AND METHODS:

Ethical Issues
Ethical clearance was got from the Institutional Animal Ethical Committee of K.M College of Pharmacy, Uthangudi, Melur road, Madurai-625 107 wide proposal number ALPHYMATHEW/MD (HOM) TNMGRTM/KA/IAEC/336.

Study Design
This is an experimental study done on ovariectomized albino rats.

Selection of Samples
Healthy female albino rats of about 90 days old, weighing about 150-220g is taken for the study.

Study Settings
Thirty (30) female Wistar albino rats are taken for the study. The rats are divided into 5 groups consisting of 6 animals each.

Intervention
Calcarea Carb is administered orally in 3 different potencies- 1X, 30 and 0/1 to 3 different groups of female Wistar albino rats respectively. The remedy is given in water dose, using distilled water, i.e., 5 drops, 3 times daily. Freshly prepared dose is administered each time.

Brief of Procedures
Female Wistar albino rats weighing about 150-220 g in the age group of about 90 days were acclimatized to the experimental room, at temperature 23+/- 2ºC, controlled humidity conditions (50-55%) and 12 hr light/dark cycle, for a period of 1 week. Animals were caged with a maximum of 2 animals each, in a polypropylene cage and fed with standard food pellets and water. After 1 week of acclimatization, the animals were randomly divided into 5 groups, containing 6 animals each. Ovariectomy was done for all the animals in all the groups following the procedure. The rats were anesthetized with a combination of ketamine HCl(80mg/kg) and xylazine (10mg/kg). The fur on the rats’ abdomens was completely removed with depilatory cream. The connection between fallopian tube and uterus was cut and the ovaries were exposed. The uterine horn was returned into the peritoneal cavity after the removal of the ovaries. The muscle incision was sutured with absorbable suture (ethicon) and skin wounds were closed bilaterally with non-absorbable suture. Prophylactic gentamycin (10mg/kg) was administered for 4 days and povidone-iodine solution was applied locally. After surgery, the rats were housed individually in polyurethane cages for a period of 1 week, to allow recovery and then were re-grouped in their home cages. The ovariectomized groups received treatment for 90 days, starting from the 15th day of ovariectomy. Group 1 was orally treated with vehicle (0.9% saline) and served as ovariectomized control. Group 2 was orally treated with estrogen (2mg/kg) daily. Estrogen tablets were supplied by Pfizer Company. Group 3 was orally treated with Calc-Carb 1X. Group 4 was orally treated with Calc-Carb 30. Group 5 was orally treated with Calc-Carb 0/1. Calcarea Carbonica 1X is prepared by trituration of Calcium Carbonate, supplied by Ganesh Chemicals, Kannur. Calcarea Carbonica 30 and 0/1 are supplied by SBL. Body weight of all the rats was measured weekly. At the end of 90 days, all the rats are deprived of food for one night.
The next day, the rats were anesthetized by ketamine HCl (50mg/kg) and blood samples were withdrawn from the retro-orbital plexuses (10) and the rats were sacrificed.

**Outcome Assessment**

**Body weight and uterine weight**

Body weight was measured at the end of treatment. Uterine weight was measured and compared with other groups were tabulated in Table 1.

**Femoral Physical Parameters**

Fresh isolated left femurs were weighed using an electronic scale. Length of the femurs was measured using a digital slide calipers. The length was measured from the proximal tip of the femur head to the distal tip of the medial condyle. Bone volume and density were measured by fluid displacement methods were tabulated in Table 2.

**Biochemical Estimation**

The levels of serum calcium, phosphorous, alkaline phosphatase and osteocalcin were measured using semi-automatic analyzer with biochemical kits were tabulated Table 3. Osteocalcin kit was supplied by Biolinkk.

**Histopathology**

The right femur was fixed in 10% formalin for 12 hr at 40°C, decalcified in 5% EDTA for 7 days and embedded in paraffin wax and cut into sagittal plane section of 5µm thickness of the femur. The sections were stained with hematoxylin and eosin and examined for histopathological changes, under a light microscope [10]. The results were compared with all the groups to see the whether Calcarea Carb and estrogen has similar effect, thus proving that Calcarea Carb is effective in treating osteoporosis were shown in Fig 1. One-way ANOVA is used to compare the means of 5 groups with respect to body weight, uterine weight, left femoral physical parameters and biochemical estimation of blood samples.

**RESULTS**

As far as the body weight is concerned, Calcarea Carb 0/1 showed the highest value (236.82 gms) among the homoeopathic potencies. For the uterine weight, Calcarea Carb 1X showed the highest value among the homoeopathic potencies.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight(grams)</th>
<th>Uterine weight (grams)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>Control</td>
<td>185.86</td>
<td>190.07</td>
</tr>
<tr>
<td>Estrogen</td>
<td>173.75</td>
<td>249.9</td>
</tr>
<tr>
<td>Calc carb 1x</td>
<td>176.48</td>
<td>231.76</td>
</tr>
<tr>
<td>Calc carb 30</td>
<td>165.25</td>
<td>215.12</td>
</tr>
<tr>
<td>Calc carb 0/1</td>
<td>166.12</td>
<td>236.82</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Groups</th>
<th>Length (mm)</th>
<th>Weight (g)</th>
<th>Volume (ml)</th>
<th>Density (g/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>35.47</td>
<td>0.68</td>
<td>0.68</td>
<td>0.99</td>
</tr>
<tr>
<td>Estrogen</td>
<td>36.16</td>
<td>0.82</td>
<td>0.73</td>
<td>1.11</td>
</tr>
<tr>
<td>Calc carb 1x</td>
<td>36.66</td>
<td>0.75</td>
<td>0.70</td>
<td>1.07</td>
</tr>
<tr>
<td>Calc carb 30</td>
<td>36.15</td>
<td>0.73</td>
<td>0.68</td>
<td>1.06</td>
</tr>
</tbody>
</table>
Calc carb 0/1 36.31 0.69 0.68 1.01

For the femoral physical parameters, Calcarea Carb 1X showed the highest value among Homoeopathic potencies.

### Table 3: Biochemical Parameters

<table>
<thead>
<tr>
<th>Groups</th>
<th>Calcium (mg/dl)</th>
<th>Phosphorous (mg/dl)</th>
<th>Alp (iu/l)</th>
<th>Osteocalcin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.5</td>
<td>7.59</td>
<td>68.17</td>
<td>1.85</td>
</tr>
<tr>
<td>Estrogen</td>
<td>7.7</td>
<td>8.23</td>
<td>104.20</td>
<td>0.65</td>
</tr>
<tr>
<td>Calc carb 1x</td>
<td>9.6</td>
<td>12.42</td>
<td>215.80</td>
<td>0.66</td>
</tr>
<tr>
<td>Calc carb 30</td>
<td>8.02</td>
<td>10.46</td>
<td>77.28</td>
<td>0.66</td>
</tr>
<tr>
<td>Calc carb 0/1</td>
<td>7.02</td>
<td>9.30</td>
<td>78.80</td>
<td>0.46</td>
</tr>
</tbody>
</table>

For the biochemical parameters, Calcarea Carb 1X ranked first in increasing the serum levels of calcium, phosphorous and alkaline phosphatase. For the serum osteocalcin, Calcarea Carb 0/1 showed the least value.

### Table 4: Histological Criteria for Osteoporosis [11]

<table>
<thead>
<tr>
<th>Score</th>
<th>Hip joint cartilage integrity</th>
<th>Structure of trabecular bone</th>
<th>Quantity of trabecular bone</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Cartilage complete</td>
<td>Normal</td>
<td>90-100%</td>
</tr>
<tr>
<td>1</td>
<td>Cartilage complete</td>
<td>Partially reduced</td>
<td>60-90%</td>
</tr>
<tr>
<td>2</td>
<td>Cartilage partially complete</td>
<td>Markedly reduced</td>
<td>30-60%</td>
</tr>
<tr>
<td>3</td>
<td>Cartilage absent</td>
<td>Absent</td>
<td>0-30%</td>
</tr>
</tbody>
</table>

![Fig 1: Histological Observations](image-url)
Group 1

- Fragments of bony tissue showing endochondral ossification.
- Marrow elements and fat observed.
- Bone edges show a “moth-eaten appearance” suggestive of increased bone resorption.
- Fragments of bone with thin trabeculae and large empty spaces suggestive of increased bone resorption seen.
- Cartilage integrity-0
- Structure of trabecular bone-1 to 2
- Quantity of trabecular bone-2
- Total score-4

Group 2

- Plenty of marrow elements
- Some of the bony fragments appear thinned and show foci of calcification.
- Bony fragments with new osteoid deposition.
- Bone fragments with foci of cartilage and irregular calcification.
- Endochondral ossification with islands of cartilage and foci of calcification.
- Skeletal muscle fragments seen.
- Cartilage integrity-2
- Structure of trabecular bone-0
- Quantity of trabecular bone-1
- Total score-3

Group 3

- Endochondral ossification
- Irregular lines of calcification
- Trabeculae appears thinned out
- Cartilage integrity-0
- Structure of trabecular bone-2
- Quantity of trabecular bone-1
- Total score-3

Group 4
Endochondral ossification noted
Osteoid deposition in whirling pattern noted.
Marrow elements seen.
Endochondral ossification seen.
Lamellar bone formation noted
Cartilage integrity-0 to 1
Structure of trabecular bone-1
Quantity of trabecular bone-0
Total score-2

Group 5
Well-formed endochondral ossification and marrow elements.
Hyaline cartilage
Woven bone maturing into lamellar bone.
Well-formed muscle and marrow elements.
Cartilage integrity-0
Structure of trabecular bone-1
Quantity of trabecular bone-1
Total score-2

Statistical Analysis
Statistical analysis is a module of data analytics. Statistical analysis involves gathering and inspecting every data sample in a set of items from which samples can be drawn. A sample, in statistics, is an illustrative selection pinched from a total population.

Analysis of Variance

<table>
<thead>
<tr>
<th>S.no</th>
<th>Anova of 5 treatment</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Body weight-final</td>
<td>5.22061</td>
<td>0.003381</td>
</tr>
<tr>
<td>2.</td>
<td>Uterine weight</td>
<td>2.76282</td>
<td>0.049756</td>
</tr>
<tr>
<td>3.</td>
<td>Left femur length</td>
<td>1.71053</td>
<td>0.179175*</td>
</tr>
<tr>
<td>4.</td>
<td>Left femur weight</td>
<td>7.78972</td>
<td>0.000321</td>
</tr>
<tr>
<td>5.</td>
<td>Left femur volume</td>
<td>1.84783</td>
<td>0.151224*</td>
</tr>
<tr>
<td>6.</td>
<td>Left femur density</td>
<td>3.25061</td>
<td>0.020566</td>
</tr>
</tbody>
</table>
DISCUSSION

Osteoporosis is a metabolic bone disease which poses a very important social and medical problem, in developed as well as in developing countries. It is becoming more of an epidemic, as there is a steady increase in the number of cases. After the age of 30, the reduction of bone mass is an unavoidable process, and consequently, alterations in the bone remodeling cycle leads to bone fragility and increased risk of bone fractures. With the continuing increase in the number of patients, osteoporosis deserves full attention and appropriate multidisciplinary approach [12]. From the study it was found that estrogen ranked the highest in average final weight, i.e, 249.9 g. Calcarea Carb 0/1 came second with a weight of 236.82 g. This was followed immediately by Calcarea Carb 1X (231.76 g) and Calcarea Carb 30 (215.15 g). This indicates that Calcarea Carb helped in maintaining the body weight after menopause and growth parameter was undisturbed by Calcarea Carb. Estrogen showed the highest value for average uterine weight which is 0.23 g. Calcarea Carb 1X ranked second with a value of 0.22 g. After menopause, with loss of estrogen, uterine atrophy is a common feature (13). Here Calcarea Carb 1X maintained uterine weight almost equal to that of estrogen, thereby protecting reproductive organs against menopausal changes. This was immediately followed by Calcarea Carb 30 (0.17 g) and Calcarea Carb 0/1 (0.16 g). This means that Calcarea Carb helped in preventing post-menopausal uterine atrophy. Estrogen ranked the highest in average left femur weight, average left femur volume and average left femur density. Calcarea Carb 1X came first in average left femur length. Reduction in bone density, bone weight and resultant bone fragility and fractures are the common phenomenon of osteoporosis [14,15]. With estrogentic loss, there is no inhibition to TNFα; TGFβ and osteoprotegerin production is reduced, contributing to increased osteoclastic activity (16). Here estrogen supplementation compensated the loss. Among homeopathic potencies, Calcarea Carb 1X showed beneficial results comparable to estrogen. Calcarea Carb 30 and Calcarea Carb 0/1 also showed beneficial effects after Calcarea Carb 1X. This proves experimentally the authenticity of Homoeopathic materia medica, which described the action of Calcarea Carb on bones years back. Calcarea Carb 1X showed the highest result among all the other groups in serum calcium, phosphorous and alkaline phosphatase levels. Calcarea Carb 30 and Calcarea Carb 0/1 follow it. This indicates that Calcarea Carb is capable of increasing and maintaining serum levels of calcium, phosphorous and alkaline phosphatase which are very much required for the bone building process [1]. For serum osteocalcin level, Calcarea Carb 0/1 showed the least value and control group showed the highest value among all the other groups. Osteocalcin being a calcium dependent biomarker will show increased value in case of bone destruction. Its value is less, when the bone destruction is minimal [17]. This study result indicates that Calcarea Carb 0/1 has minimum bone destruction whereas control group has maximum destruction compared to all the other groups. This also indicates that Calcarea Carb 0/1 has effectively utilized the serum calcium, phosphorous and alkaline phosphatase levels for bone building process and caused very minimal bone destruction. From histopathological estimation of right femur, it is evident that reduced osteoporosis, better quality of cartilage and marrow are found in Calcarea Carb 0/1. It is immediately followed by Calcarea Carb 30, Calcarea Carb 1X and estrogen. Control group has maximum osteoporotic changes. Here Calcarea Carb 1X has elevated the physical and biochemical parameters, but has not converted or used serum calcium and phosphorous for deposition and bone formation which is evident from serum levels of osteocalcin and histopathological findings. This indicates
that the action of Calcarea Carb 1X is supplementary in nature due to its physiological or material or crude dose. Calcarea Carb 30 is also found to be effective in controlling osteoporosis, meeting the needs of bone—physically, serologically and histologically. Calcarea Carb 0/1 has not only elevated the physical and biochemical parameters but also has effectively used calcium and phosphorous for deposition and bone formation which is confirmed from the serum osteocalcin and histological observations. This indicates that the dynamic Calcarea Carb 0/1 is the best potency for the bone formation and its action is not supplementary, but curative, not in terms of totally restoring the bony architecture but in terms of effective osteoblastic activity.

CONCLUSION

Calcarea Carb is effective in controlling osteoporosis in ovariectomized albino rat model of osteoporosis in 3 different potency scales i.e, 1X, 30 and 0/1. Calcarea Carb 0/1 is effective in controlling osteoporosis by using the elevated biochemical parameters, beneficially for osteoblastic activity. It has also taken care of the bone physically and histologically. So Calcarea Carb 0/1 is the best potency for controlling osteoporosis as per the study. Calcarea Carb 30 is the next effective potency for controlling osteoporosis, which is evident from the physical, biochemical and histological parameters. Calcarea Carb 1X due to its physiological or material nature, has elevated the physical and biochemical parameters to the maximum, but has not showed remarkable changes in bony architecture. This confirms that its action is supplementary in nature. The action of Calcarea Carb is comparable to that of estrogen in elevating physical and biochemical parameters and acted more than estrogen, in histological parameter and serum osteocalcin parameter especially in dynamic doses of Calcarea Carb 30 and Calcarea Carb 0/1.

ACKNOWLEDGEMENTS

We thank Global Homoeopathic Foundation for financial support.

REFERENCES

9. Suresh D. A Retrospective Case Series Study To Understand The Evolution Of Calcarea Carb in Clinical Situations. The Tamil Nadu Dr.M.G.R Medical University; 2017.


