Original Article

An ayurvedic proprietary herbal preparation, Calci-7, prevents ovariectomy-induced osteoporosis in rats

Khushboo G. Faldu, Harika Duvva, Jigna S. Shah, Prabooth V. Shah[1], Snehal S. Patel

Department of Pharmacology, Institute of Pharmacy, Nirma University, [1]Department of Pharmacology, Virgo Uap Pharma Pvt. Ltd., Moraiya, Ahmedabad, Gujarat, India

Abstract

Introduction: CALCI-7 (CA) is an ayurvedic proprietary medicine, used to treat osteoporosis in postmenopausal women. However, scientific proof for its anti-osteoporotic effect and mechanism of action remain unclear due to which present study was under taken. **Materials and Methods:** Ovariectomy was performed to induce osteoporosis. Rats were given CA 100 mg/kg, p.o. and estrogen 0.0563 mg/kg, i.m. once a day from day 1 to day 42. Animals were weighed weekly for body weight variation. At the end of 42 days treatment, urine and the blood samples were collected for analysis of calcium, phosphorus and alkaline phosphatase. Immediately, after sample collection, the uterus was carefully removed and weighed. Results were analysed using ANOVA. **Results:** CA treatment to ovariectyomized (OVX) rats showed significant improvement in body weight, increased levels of calcium and phosphorus levels in serum while levels were found to be decreased in urine as compared to OVX group. The significant increase in uterine weight and femur length were observed in treatment groups. In the histopathological examination bone structure showed a normal structure with lacuna and various lamellae without any damage or porosity in bone in the formulation and estrogen-treated group. **Conclusion:** The present findings are strongly suggestive that CA possesses comparable efficacy to estrogen and thus can be useful in the postmenopausal osteoporosis.

Keywords: Anti-osteoporotic, ayurvedic formulation, estrogen, osteoblast, ovariectomy

Introduction

Osteoporosis, a bone remodeling disorder is characterized by the reduction in bone mass with distortion of the microarchitecture, characterized by the condition of weak and porous bones. It is the most frequent disease and a major health problem for postmenopausal women.[1] The main pronominal factor for bone loss in postmenopausal women is estrogen deficiency. Standard therapies include anti-resorptive drugs that decrease bone loss, e.g., bisphosphonates, calcitonin, selective estrogen receptor modulators, calcium and anabolic agents that increase bone formation.[2,3] These treatments are costly, with the exception of the combination of calcium and Vitamin D and exceed the economic resources of patients in many countries and are associated with severe side effects.

Ayurveda seems to be effective in addressing the above-mentioned limitations of the conventional therapies. Ayurvedic prorietary formulation CALCI-7 (CA) is already there in Indian market and also recommended by ayurvedic medical practitioners for treatment of osteoporosis. However, the anti-osteoporotic properties of this formulation have not been subjected to any scientifically controlled investigations so far. Therefore, the objective of this study was to evaluate the effect of CA in reducing postmenopausal osteoporosis induced by ovariectomy in rats.

Materials and Methods

Materials

The ingredients of the herbomineral formulation were authenticated and voucher specimen were preserved by Virgo UAP Pharma Pvt. Ltd. Sanand, Ahmedabad, Gujarat, India. The tablet was formulated by Virgo UAP Pharma

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Address for correspondence: Dr. Snehal S. Patel, Department of Pharmacology, Institute of Pharmacy, Nirma University, Ahmedabad - 382 481, Gujarat, India.
E-mail: snehalpharma53@gmail.com

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Pvt. Ltd based on ingredient specification of the official Ayurvedic Formulary of India [Table 1]. CA is composed of *Jaharmohara bhasma, Shukti bhasma, Mukta Shukti bhasma, Shankh bhasma, Kapardi Bhasma, Godanti Bhasma, Mandur Bhasma* and *Asthisanhari*. The Acute toxicity study has shown that formulation is safe up to 2000 mg/kg dose as per OECD Guidelines, 2004.

Experiment animals

Healthy female Wistar rats (250–300 g) were procured from Torrent Research Centre, Gandhinagar, Ahmedabad. Animals were grouped and housed in the animal house of Institute under controlled atmospheric conditions i.e., temperature 23°C ± 2°C, relative humidity 55% ± 5%, and photo-schedule (12 h light and 12 h dark). Food and purified water *ad libitum* were freely accessible to the animals. Institutional Animal Ethics Committee has approved all the experiments and protocols described in the study. One week acclimatization period was followed before starting the experiment. The pharmacological work was carried out as per approved protocol (IPS/PCOL/CONS12–13/1003).

Experimental protocol

Rats were divided into four groups of six animals each i.e., Sham-operated group, Ovariectomized (OVX) control group, OVX treated with CA (100 mg/kg) group, OVX treated with estrogen (0.056 mg/kg) [Table 2]. Ovariectomy was performed to induce osteoporosis.[4,5] Treatment schedule comprised of once daily dosing from day 1 to day 42. Every week animal's body weight was measured for determination of weight gain. Body weight of animals was measured weekly.

Table 1: Pharmaceutical preparation of CA

Ingredients	Botanical name/ English name	Composition per 250 mg
Jaharmohara Bhasma	*Calx serpentine*	15 mg
Shukti Bhasma	*Oyster Shell*	30 mg
Mukta Shukti Bhasma	*Pearl oyster*	40 mg
Shankh Bhasma	*Conch shell*	60 mg
Kapardi Bhasma	*Purified cowries*	80 mg
Godanti Bhasma	*Gypsum*	80 mg
Mandur Bhasma	*Iron oxide*	30 mg
Extract of .Asthisanhari	*Cissus quadrangularis* Linn.	60 mg

CA: CALCI-7

Table 2: Animal grouping and dose of administration

Group	Treatment	Number of animals
1	Sham operated receive vehicle (0.5% Na-CMC)	8
2	Ovariectomized control receives vehicle (0.5% Na-CMC)	8
3	Formulation in 0.5% Na-CMC (100 mg/kg) in ovariectomized rats	8
4	Estrogen (0.0563 mg/kg) in ovariectomized rats	8

At the end of 42 days, all the rats were fasted overnight. On a succeeding day, urine and blood samples from retro-orbital plexus were collected for analysis of calcium, phosphorus and alkaline phosphatase (ALP).[6,7] Immediately, after collecting the urine and blood samples, animals were sacrificed, the uterine horn was isolated removing all the surrounding fat and weighed immediately. The femur and lumbar vertebra were isolated, its thickness and length were measured and subjected to the compression test and histopathological evaluation. Femur bone was ashed in a furnace at 800ºC for 12 h and calcium content was measured using an atomic absorption spectrophotometer.

Statistical analysis

Values are expressed as mean ± standard error of the mean (SEM). The results were analyzed using one-way factorial analysis of variance followed by Tukey's multiple comparison test using GraphPad Prism 5 software (GraphPad Software, Inc. CA, USA). The value of $P < 5\%$ ($P < 0.05$) was considered as statistically significant.

Results

Effect of CALCI-7 on physical parameters

Disease control i.e., OVX group showed a reduction in body weight together with decreased uterine weight significantly as compared to the sham operated group. CA and estrogen-treated animals showed a statistically significant rise in uterine weight; while there was an in-significant increase in body weight was observed in the treatment group [Table 3].

Effect of CALCI-7 on the weight, length, hardness and calcium content of femur bone

OVX group showed a significant reduction in weight, length and hardness of femur bone as compared to the sham operated group. CA and estrogen-treated animals showed a statistically significant rise in all these physical parameters, while there was in-significant increase in body weight observed by treatment. We have also estimated calcium content in femur bones of OVX group which was found to be significantly decreased, and improved after treatment with CA as well as estrogen [Table 3].

Effect of CALCI-7 on serum biochemical parameters in OVX rats

Serum calcium and phosphorus levels were significantly reduced as compared to the sham-operated group while serum ALP levels showed an increase in comparison to the sham operated group. CA and estrogen-treated groups showed recovery in serum calcium and phosphorus levels as compared to OVX group. Serum ALP levels reduced significantly in CA and estrogen-treated groups [Figure 1].

Effect of CALCI-7 on urine biochemical parameters in OVX rats

Urine calcium and phosphorus levels were found to be increased in OVX group as compared to the sham operated group, while significant decrease in urine calcium and phosphorus levels was seen in CA and estrogen-treated groups [Table 4].

Table 3: Effect of CA on physical parameters

Parameters	Sham operated group	DC group	DTCA group	DTE group
Body weight gain (g)	24.83±1.95	17.5±1.26*	20.83±3.09	19.5±1.26
Weight of uterus (g)	0.69±0.067	0.402±0.037	0.820±0.10#	0.675±0.05#
Weight of the femur (g)	0.577±0.02	0.318±0.01*	0.477±0.02#	0.476±0.02#
Length of femur (mm)	3.11±0.02	2.363±0.06*	3.10±0.016#	3.10±0.018#
Thickness of femur (mm)	5.09±0.14	4.18±0.04*	5.10±0.06#	4.99±0.37#
Hardness of femur (kilopounds)	4.92±0.19	2.75±0.10*	4.49±0.16#	4.65±0.30#
Percentage calcium in bone	1.30±0.058	0.42±0.130*	1.03±0.031#	1.00±0.138#

Values are expressed as mean±SEM of 6 animals. *Significantly different from normal control (P<0.05), #Significantly different from DC (P<0.05), The results were analyzed using one-way factorial analysis of variance followed by Tukey's multiple comparison test using Graphpad Prism 5 software. DC: Disease control, DTCA: Diseased animals treated with CA (100 mg/kg), DTE: Diseased animal treated with estrogen (0.0563 mg/kg), SEM: Standard error of mean, CA: CALCI-7

Table 4: Effect of CA on urine biochemical parameters

Parameters	Sham operated group	DC group	DTCA group	DTE group
Urine calcium (mmol/L)	18.14±0.19	20.25±0.10*	18.88±0.17#	19.53±0.09#
Urine phosphorus (mmol/L)	36.67±9.55	106.70±9.89*	27.17±3.06#	72.50±8.03#

Values expressed as mean±SEM of 6 animals each. *Significantly different from normal control (P<0.05), #Significantly different from DC group (P<0.05), The results were analyzed using one-way factorial analysis of variance followed by Tukey's multiple comparison test using Graphpad Prism 5 software. DC: Disease control, DTCA: Diseased animals treated with CA (100 mg/kg), DTE: Diseased animal treated with Estrogen (0.0563 mg/kg), SEM: Standard error of mean, CA: CALCI-7

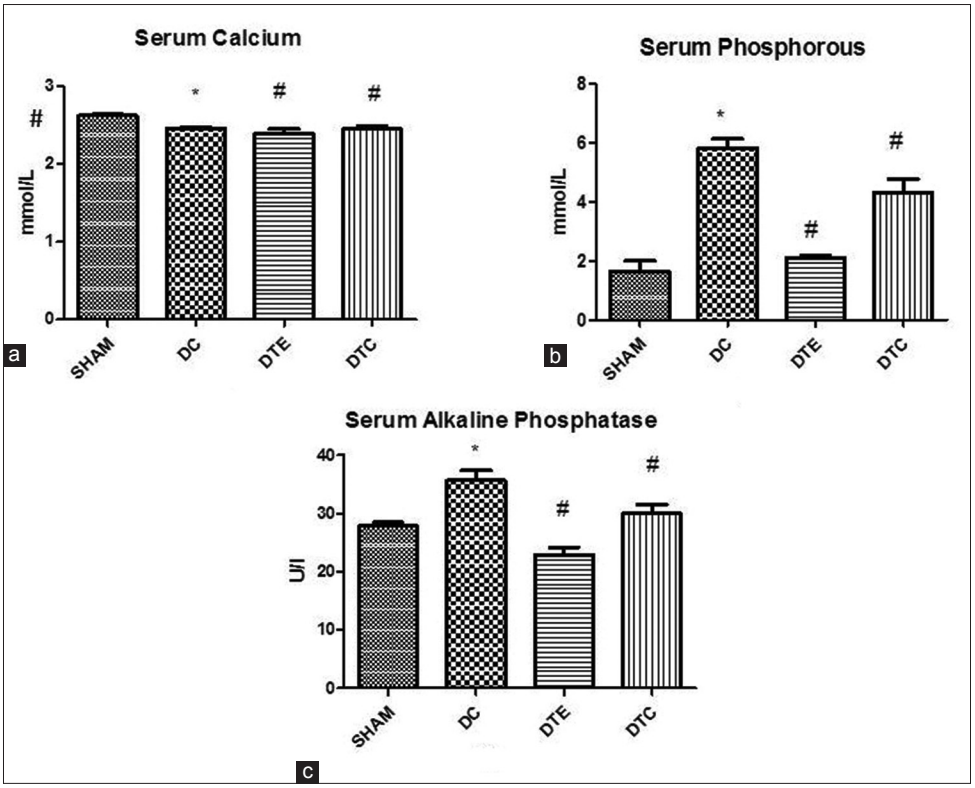

Figure 1: (a-c) Effect of CALCI-7 on serum biochemical parameters in Ovariectomized rats: Serum Calcium, Serum Phosphorus, Serum alkaline phosphatase. Values expressed as mean ± standard error of the mean of 6 animals each, *Significantly different from normal control ($P < 0.05$), #Significantly different from disease control group ($P < 0.05$). SHAM-sham operated, DC-Disease control, DTCA-Diseased animals treated with CALCI-7 (100 mg/kg), DTE-Diseased animal treated with Estrogen (0.0563 mg/kg)

Effect of CALCI-7 on histopathological evaluation of OVX rats

In histopathological examination, normal control showed a normal bone structure with lacuna and various lamellae without any damage or porosity in bone. While, OVX group showed vacuolization with chondrocyte presence in cartilage portion of the bone. In OVX animal treated with CA, bone

structure showed a normal structure with lacuna and various lamellae without any damage or porosity in bone. Similar histoarchitecture was also observed in the estrogen-treated group [Figure 2].

Discussion

Osteoporosis is the structural failure of the skeleton and causes an increased risk of fracture. Low-energy trauma can cause fractures due to low bone mass, microarchitectural deterioration of bone tissue and increased bone fragility.[8] Anti-osteoporotic agents are presently directed to two basic processes of bone viz., bone remodeling and bone resorption.[9,10] The most common type of osteoporosis is the bone loss associated with ovarian hormone deficiency at menopause. Especially, in women the estrogen deficiency, results in increased plasma calcium levels as a result of increased bone resorption.[11] Thus, alternative approaches for managing osteoporosis are needed and in this regard, we had evaluated the anti-osteoporotic activity of CA in ovariectomized rats.

The ovariectomized rat exhibited most of the characteristics of human postmenopausal osteoporosis.[12] The reduction in body weight in OVX group was observed and in treatment groups, increase in body weight was observed as compared to sham-operated groups. Vaginal atrophy is the characteristic feature of ovariectomized rats.[13,14] In present study uterine weight was found to be decreased in OVX group as compared to the sham operated group of animals and the treatment with estrogen and CA showed statistically significant increase in uterine weight. Estrogen is reported to prevent bone loss by suppressing the rate of bone turnover involving the depression of both osteoclastic and osteoblastic bone formation.[15-17] The results of study showed reversal of uterine atrophy suggestive of estrogenic activity of formulation. The formulation contains *Cissus qadrangularis* Linn. as one of the ingredients which has been reported to have estrogenic activity.[18] Thus, the probable mechanism involved may be the estrogenic activity of formulation due to *Cissus qadrangularis* Linn.

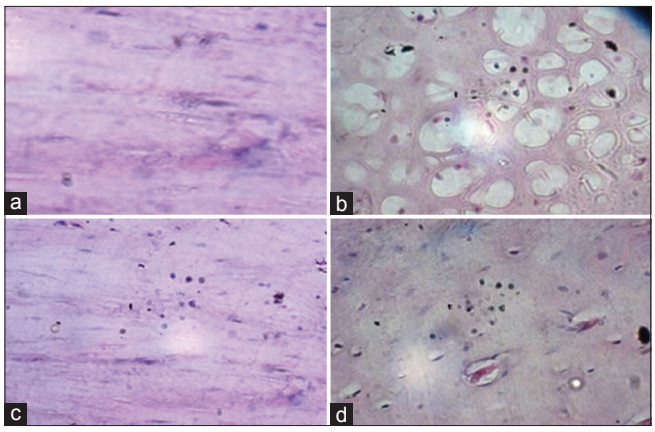

Figure 2: (a-d) Effect of CALCI-7 on histopathological evaluation of Ovariectomized rats: Normal control, Disease control, Diseased treated with CALCI-7, Diseased treated with estrogen

In our study, the bone loss in ovariectomized rats was significant throughout the study in terms of weight, length and hardness of femur bone as compared to the sham operated group. The estrogen deficiency in OVX rat is associated with a higher rate of bone turnover where the increase in bone resorption is relatively greater than the increase in bone formation.[19,20] Femur bone is more sensitive to high turnover bone loss.[21-22] CA and estrogen-treated animals showed statistically significant improvement in all these physical parameters. Estrogen and CA treatment showed a beneficial effect on the ash calcium content which was found to significantly decreased in OVX group. It has been reported that bhasmas are a natural source of rich calcium widely used in traditional system of Indian medicine as a supplement in the treatment of a variety of bone metabolic disorders associated with calcium deficiency.[23] CA is composed of seven different *Bhasmas (J. Bhasma, S. Bhasma, M. Shukti Bhasma, S. Bhasma, K. Bhasma, G. Bhasma, M. Bhasma)* which is a rich source of calcium. Therefore, the beneficial effect of formulation on all physical parameters may be due to the presence of calcium present in the formulation.

Significant reduction was observed in serum calcium and phosphorus levels of OVX group following ovariectomy which is indicative of enhanced bone fragility while the significant increase in the ALP levels following ovariectomy is suggestive of increased bone turnover which may account for bone disorders.[24-26] Our investigation showed that treatment with CA was able to prevent an increase in urine calcium and phosphorus levels as compared to OVX group which was comparable to estrogen. Results may be a suggestive factor for enhanced bone formation that may account for bone disorders.

ALP is a biomarker of osteoblastic and osteoclastic activities.[27,28] The serum ALP levels were found to be increased in OVX group which was reduced in treatment groups. Thus, results of all biochemical parameters further confirm anti-osteoporotic property of formulation.

Histopathological examinations reveal that the OVX group treated with CA showed ossification, mineralization and calcified cartilaginous deposit and enhanced osteoclastic activity; these observations are evidence for a marked restoration of bone loss. This observation indicates the potential protective action of CA and these actions may be due to an increased bone formation with a reduction in bone resorption.[18,28]

Conclusion

Thus, on the basis of above results we can conclude that CA have significant anti-osteoporotic effect in postmenopausal osteoporosis and the study has laid down the scientific proof for treatment of osteoporosis. Thus, it is suggestive that CA can be considered as safe supplementary therapy for effective and long term management of osteoporotic patients.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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हिन्दी सारांश

लौह की कमी से होने वाले एनीमिया के प्रबंधन में प्रयुक्त विभिन्न आयुर्वेदिक औषधि योग– एक व्यवस्थित समीक्षा

जन्मेजय समल

लौहतत्व की कमी से होने वाला एनीमिया (आई.डी.ए.) एक वैश्विक स्वास्थ्य संकट है । विश्व स्वास्थ्य संगठन के अनुसार होने वाले कुल एनीमिया रोगियों में आधा, आई.डी.ए. के कारण होता है। कई आयुर्वेदिक औषध योग आईडीए (आई.डी.ए.) के प्रबंधन के लिए उपलब्ध हैं। इस संदर्भ में, आईडीए के नियंत्रण के लिए आयुर्वेदिक दवाओं की भूमिका को समझने के लिए एक व्यवस्थित शोध समीक्षा की गई। इस उद्देश्य के लिए लेख पबमेड और स्वतः खोज प्रक्रिया के माध्यम से प्राप्त किये गये। चुने गये ३७ लेखों में से अंत में १० लेखों का समीक्षा के लिए चयन किया गया।१० अध्ययन में से ३ अध्ययन (n = १०) विशेष रूप से गर्भवती महिलाओं के लिये, २ अध्ययन (n = १०) विशेष रूप से बाल आयु समूह, १ अध्ययन (n = १०) बुढ़ापे की एनीमिया पर विशेष रूप से आधारित थे और ४ अध्ययन (n = १०) सामान्य व्यक्तियों पर आधारित थे । अधिकांश आयुर्वेदिक दवाओं का प्रभाव एलोपैथिक दवाओं से बेहतर था और इनमें से किसी में भी अनुपयुक्त परिणाम जैसे कि लौह लवण द्वारा प्राप्त होते हैं, नहीं पाये गये। व्यक्तिपरक और हिमेटोलोजिकल निष्कर्ष में सभी शोध में प्रयुक्त आयुर्वेदिक दवाओं के पक्ष में सांख्यिकीय दृष्टि से सार्थक परिणाम पाये गये। इन छह विभिन्न दवाओं में से सर्व-ज्वरहरलौह को आई.डी.ए. के लिए उत्तम दवा के रूप में, क्योंकि एक शोध के आधार पर उसके प्रयोग से हीमोग्लोबिन निर्माण उच्चतम प्राप्त हुये हैं (०.१६ g/dl/day)। इसके अलावा वर्तमान में पुनर्नवादि मण्डूर एक एनीमिया नियंत्रण औषध के रूप में स्थापित किया जा सकता है । इसको राष्ट्रीय ग्रामीण स्वास्थ्य मिशन के माध्यम से प्रचार मिला है और मान्यता प्राप्त सामाजिक स्वास्थ्य कार्यकर्ता (ए.एस.एच.ए.) की किट में भी उपलब्ध है। इससे यह निष्कर्ष निकाला गया कि शोध से स्थापित इन सभी पांडुहर आयुर्वेदिक औषध योगों का प्रयोग वर्तमान में प्रयुक्त एलोपैथिक दवाओं के साथ पांडु चिकित्सा में उचित रूप से किया जा सकता है।