

Quality of bone repair in ovariectomized rats with bone lesions treated with phytotherapeutic and homeopathic *Arnica montana*

Qualidade do reparo ósseo em ratas ovariectomizadas, lesionadas e tratadas com *Arnica montana* fitoterápica e homeopática

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ABSTRACT

Objective: National and international data show that in the next years the elder population tends to have an exponential increase becoming to be significantly higher than other ages. Among the frequent diseases in the elderly, osteoporosis is a disease that decreases bone mass and deteriorates bone structure causing fragility and a high risk of fracture. This study aimed to evaluate the effect of phytotherapeutic and homeopathic *Arnica montana* on bone repair quality and its possible use in the treatment of bone fracture in patients with osteoporosis. **Material and Methods:** This experimental study was performed in Wistar adult female rats divided in 4 groups according to the following treatments: ovariectomized treated with homeopathic *A. montana* 6CH (OVZ 6CH); ovariectomized treated with phytotherapeutic *A. montana* (OVZ TM); ovariectomized with placebo (OVZ PL) and rats with sham surgery and placebo (Sham PL). In a period of 45 days after ovariectomy or sham surgery, all animals got a monocortical lesion. They were euthanized after 6, 12, 18 and 28 days and had the tibiae removed to evaluate dimensions and bone repair by radiographic density, biomechanical test and scanning electronic microscopy (SEM). Data were analyzed by ANOVA and TUKEY tests ($p < 0.05$). **Results:** Results showed that treatment with *Arnica* 6CH was better than *Arnica* TM considering bone resistance and bone repair quality. **Conclusion:** This study concluded that treatment with homeopathic *A. montana* was

RESUMO

Objetivo: Dados estatísticos nacionais e mundiais demonstram que o número de indivíduos idosos tende a crescer de maneira exponencial nos próximos anos, tornando-se uma população significativamente e proporcionalmente maior que as demais. Entre as doenças mais comuns nessa faixa estaria encontra-se a osteoporose, doença que reduz a massa esquelética e deteriora a microarquitetura do osso causando fragilidade e aumentando o risco de fraturas. **Material e Métodos:** Este estudo teve como proposta avaliar o efeito da *Arnica montana* fitoterápica e homeopática no reparo de lesões ósseas, visando uma possível forma de tratamento de fraturas na vigência da osteoporose. Os ensaios experimentais foram realizados em ratas Wistar adultas, divididas em quatro grupos: ovariectomizado tratado com *Arnica montana* 6CH homeopática (OVZ 6CH); ovariectomizado tratado com extrato fitoterápico de *Arnica montana* (OVZ TM); ovariectomizada tratado com placebo (OVZ PL) falsa cirurgia de ovariectomia e tratado com placebo (Sham PL). Após 45 dias da cirurgia sham ou de ovariectomia, foi realizada uma lesão óssea monocortical. Os animais foram eutanasiados após 6, 12, 18 e 28 dias e as tíbias retiradas para avaliação das suas dimensões e análise do reparo ósseo através de densidade óssea radiográfica, ensaios biomecânicos e microscopia eletrônica de varredura (MEV). Os dados do teste de flexão foram submetidos à análise estatística pelos testes de ANOVA e Tukey ($p < 5\%$). **Resultados:** Conclui-se que o tratamento com *arnica* 6CH foi melhor que o com *arnica* TM quanto à resistência e à qualidade de regeneração óssea, enquanto a TM mostrou melhor valor de densidade óptica. **Conclusão:** Esse estudo concluiu que o tratamento com *Arnica montana* homeopática foi mais eficiente que com

more efficient than with phytotherapeutic *Arnica* in bone regeneration in rats with osteoporosis.

KEYWORDS

Arnica montana; Homeopathy; Osteoporosis; Phytotherapy; Radiographic bone density.

Arnica fitoterápica na regeneração óssea de ratas com osteoporose.

PALAVRAS-CHAVE

Osteoporose; *Arnica montana*; Homeopatia; Fitoterapia; Exames radiográficos.

INTRODUCTION

Until 2025, according to the World Health Organization [1], Brazil will be the sixth country in the world in number of elderly. The increase in average life expectancy has sharply risen; however, it needs to be accompanied by improvement or maintenance of health and quality of life [2].

A common disease in this age group is osteoporosis, a metabolic disease characterized by reduced skeletal mass and deterioration of microarchitecture, resulting in increased bone fragility and, consequently, increased fracture risk [3-7].

Alternative therapies such as homeopathy and phytotherapy are used to treat various diseases due to their therapeutic effects. It is believed that they have fewer side effects and lower financial costs compared to allopathic therapy.

Arnica montana is a herbaceous perennial plant used in the treatment of contusions and wounds since ancient times. It is a popular remedy also used in complementary medicine to treat contusions, trauma-associated swelling, pain, inflammation and wounds. There are few scientific studies evaluating its effects on osteoporosis and wound healing in the presence of osteoporosis. Some experimental evidence in laboratory animals found an anti-inflammatory action of *A. montana* [8].

The action of *A. montana* as a promoter of healing by means of increasing the expression of several genes and some of these ones are key

regulators for tissue remodelling, inflammation and chemotaxis [8].

According to Sutovska, topic *A. montana* is strongly recommended in veterinary phytotherapy, for treatment of inflammation of tendons and joints and also for treatment of wounds of skin and mucous membranes, eczema and skin inflammation. It is also known for its biologically active acidic polysaccharides and glycoconjugates [9].

Taking into consideration all the beneficial activities of this plant, this study aimed to evaluate the effect of *A. montana* as phytotherapeutic and homeopathic medicine, in the quality of bone repair in rats with osteoporosis. In this study it was hypothesized that homeopathic *A. montana* 6CH improves bone repair in osteoporotic rats due to its higher action mechanism.

MATERIAL AND METHODS

This study was conducted according to the Ethical Principles in Animal Experimentation adopted by the Brazilian College of Animal Experimentation (COBEA) and was approved by the Ethics Committee of São Paulo State University (UNESP), Institute of Science and Technology, under the protocol number 023/2009-PA/CEP.

Ninety rats (*Rattus norvegicus*, Albinus variation, Wistar) weighing approximately 300 g were used. They were 90 days old. The animals were submitted to ovariectomy and after 30 days the surgical procedures were performed. They received general anesthesia using aqueous 2% 2-ethyl (2,6-xylylidine) -5,6-dihydro-4H-

1,3-thiazine (Bayer Anasedan[®], São Paulo-SP, Brazil) associated with Ketalina base (Dopalen[®]-Agribrands of Brazil Ltda) at a ratio of 0.8:0.5 mL and intramuscularly injected in a dose of 0.1 mL/100 g of animals after hair removal and skin antisepsis in both tibias, incision was made approximately 1.5 cm in skin and muscle in the proximal tibia third region. After exposure of bone tissue, a monocortical surgical defect with 2.5 mm diameter was performed until the bone marrow limit, under constant irrigation with sterile saline. Suture plans were held with Silk 4-0 (Ethicon[®], Brazil). Each group had its subgroups of six animals each and were divided according to the treatments:

- Sham PL group - rats with sham surgery and treated with placebo;
- OVZ PL group - ovariectomized rats treated with placebo;
- OVZ 6CH group - ovariectomized rats treated with homeopathic *A. montana* 6CH dissolved in 0.03381402 fl oz of water/animal/day orally (Gavage);
- OVZ TM group - ovariectomized rats treated with *A. montana* mother tincture (TM) phytotherapeutic, 0.4057683 fl oz TM available for 24 h;

Homeopathic and phytotherapeutic were purchased at Pharmacia Antiga - São José dos Campos, SP – Brazil.

The animals did not receive anti-inflammatory and antibiotic after surgery to avoid possible medication interaction. All rats were euthanized, in groups of six animals after 6, 12, 18 and 28 days after the beginning of the tests. General anesthesia (Anasedan[®] and Dopalen[®]) was used in overdose for euthanasia.

Tibias were radiographed by Digital System CCD type (Direct Digital Intraoral RVGui, version 5.0). During the X-ray analysis, tibias were positioned with the surgical defect facing the sensor, centralized and fixed, so that there was no distortion and overlapping images. The analysis of bone density was performed with the

division of the histogram in 4 equal parts which comprised an analysis by color graduations in gray scale composed by a table which varies from 0 to 255 pixels, being black (0 value) the initial and white (500 value) the final color.

Morphological analysis was done through scanning electron microscopy (SEM) Zess EVO[®] MA10 in backscattered electron mode (BSE). The right tibias were submitted to a previous preparation, being embedding in cold resin (Bakelite), sanded, polished and then cleaned. A thin layer of gold was sputtered onto the surface, with the purpose of increasing the conductivity of electrons and then, improving the quality of SEM images.

Previously to biomechanical test of the right tibia, measurements as overall size and thickness of the distal region using a digital caliper Mitutoyo[®] were carried out. All right tibias with surgical defect suffered mechanical stress so that the results were compared among the groups, followed by evaluation of bone regeneration in the flexural strength variation. Biomechanical properties of tibias were determined by the evaluation of resistance and flexure by means of three point bending test, using universal testing machine EMIC SL 2000. The test was performed in the distal cylindrical portion of the tibia, in an area with 15 mm in length. The load was applied at its midpoint until complete fracture of the tibia. Data of biomechanical tests were submitted to ANOVA test. A significance level of 5% was adopted.

RESULTS

The results of analysis of bone quality of the groups with their respective treatments are shown in figure 1. All results were used for comparison between groups with operated (OVZ) and not operated (SHAM) rats, as well other groups operated and treated with *A. montana* and tincture 6CH. All groups were evaluated at 6, 12, 18 and 28 days.

After 6 days of surgical defect, the images showed low bone recovery of SHAM PL and OVZ PL groups. Images of OVZ TM group suggest a

higher bone growth than OVZ 6CH, with the presence of parallel trabecular bone and with large porosities. On the other hand, OVZ 6CH had a thinner superficial layer in which more trabecular bone was observed.

On the 12th day, a significant increase of bone tissue for all groups could be noticed. SHAM PL group presented a good bone growth, both in the defect area as well as in its depth, occupying the medullar area. OVZ PL group bone neoformation showed a disorganized trabecular bone occupying the area of the lesion and also reaching the bone marrow. In OVZ TM group, the bone regeneration consisted of a bone layer on its surface and a thinner layer in its depths, but large pores between the layers were observed. The tibia of OVZ 6CH group presented a thicker and compact bone on the surface, but with pores and trabecules in the depth.

After 18 days, it was verified higher fulfilling of the lesion area in SHAM PL, OVZ PL

and OVZ 6CH groups than in the OVZ TM group, which showed depression in the central area defect. In the SHAM PL and OVZ PL groups, a smaller thickness than the old cortical bone was observed, but the trabecular bone growth was occupying part of the marrow with expansion to the surface of the lesion, in SHAM PL. OVZ 6CH group showed compact bone fulfilling the entire extension of the lesion, near the old cortical thickness. OVZ TM group exhibited only trabecular bone in all extension.

After 28 days, different patterns were verified in bone regeneration. In SHAM PL group, bone neoformation was composed by a thin lamellar bone layer. In OVZ PL group, bone growth occurred with high porosity at the deepest part of surgical defect and disorganization of the trabeculae. In OVZ TM and OVZ 6CH groups, lamellar compact bone was observed, forming a thin layer.

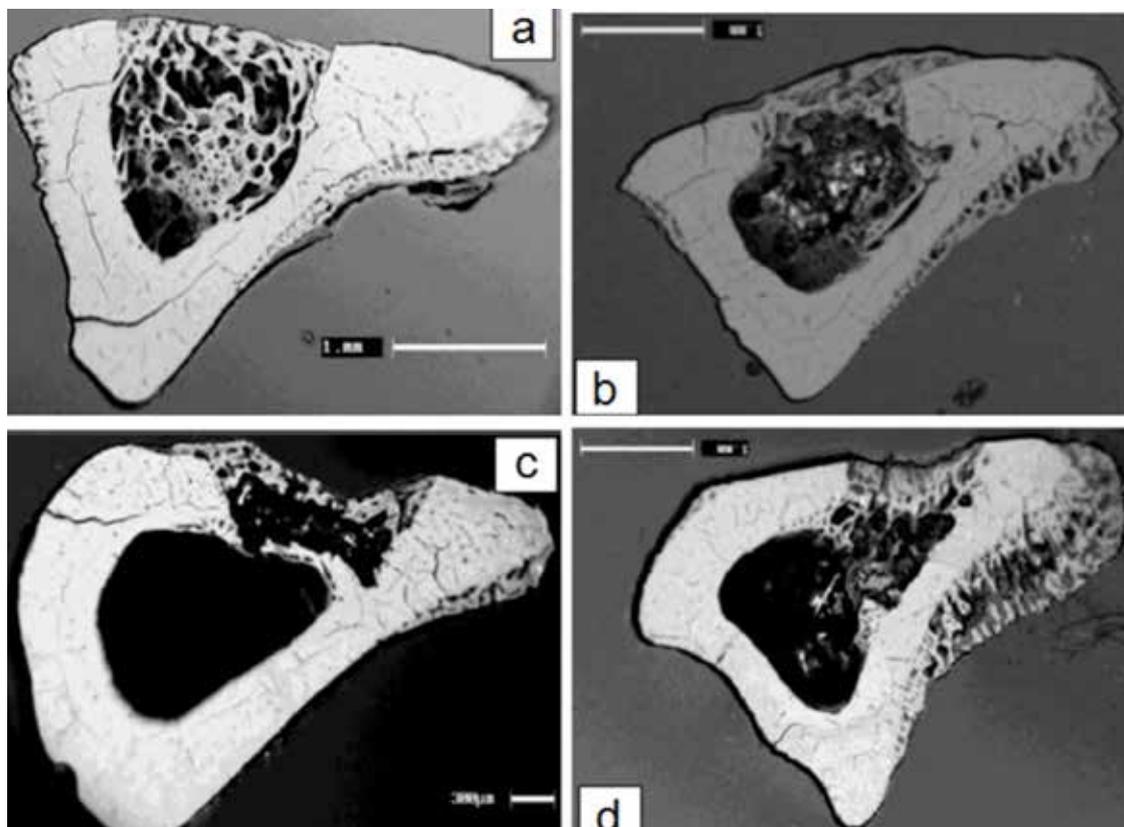


Figure 1 - Scanning electron micrograph of a transversal section: a) Sham PL; b) OVZ PL; c) OVZ TM; d) OVZ 6CH.

The results of analysis of images of digital radiography of optical density, tibia dimensions, biomechanical resistance in three point bending test are shown in tables 1 and 2. The means of the factors treatment, time of repair and interaction between them were statistically analyzed by two way ANOVA test. The results for the analysis of dimensions of the tibia revealed no statistically significant difference ($p > 0.05$) (Table 1). On the other hand, the analysis of images of digital radiography of optical density and biomechanical resistance in three point bending test revealed statistically significant difference ($p < 0.05$) (Table 2). The smallest optical density was observed in the OVZ PL group, at 28 days, differing from Sham PL group ($p < 0.05$), which exhibited higher values. OVZ PL and Sham PL groups presented statistical difference intergroup between the periods of 6 and 28 days ($p < 0.05$). Regarding biomechanical resistance, there was statistically significant difference among the groups ($p < 0.05$) (Table 2), being that Sham PL, at 28 days, presented the highest value and, OVZ PL, at 18 days, showed the smallest value.

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DISCUSSION

Because of advances in medicine, social and economic conditions of people, an increased in life expectancy and, consequently, an increase in the number of elderly can be observed nowadays. These aspects are followed by an increase in the incidence of typical diseases of this age group, such as osteoporosis [2]. Osteoporosis is a chronic degenerative disease in which bone reabsorption predominates over bone formation, making bones more susceptible to fractures [3-7].

The treatment of osteoporosis and its complications represent a significant socio-economic impact for patients and for health sector. In addition to medications, it is necessary to equip hospitals and qualify professionals for this specific service [2].

Alternative therapies such as homeopathy and phytotherapy are widely used due to its therapeutic effects and it is believed that they may cause fewer side effects. Besides, both have lower financial costs compared to allopathic therapy. However, there are still few scientific reports on its use in bone diseases.

Table 1 - Effects of treatments on dimensions (mm) of tibia

Total length tibia	Sham PL	OVZ PL	OVZ6CH	OVZ TM
6 days	40.3 ± 0.01	39.6 ± 0.02	39.2 ± 0.02	40.0 ± 0.01
12 days	39.4 ± 0.40	40.2 ± 0.04	39.6 ± 0.37	39.8 ± 0.40
18 days	39.6 ± 0.27	39.0 ± 0.20	40.3 ± 0.29	38.6 ± 0.25
28 days	41.0 ± 0.27	39.7 ± 0.20	40.4 ± 0.29	39.3 ± 0.25
Tibia diameter	Sham PL	OVZ PL	OVZ 6CH	OVZ TM
6 day	2.30 ± 1.9	2.25 ± 2.61	2.28 ± 4.05	2.36 ± 3.95
12 days	2.2 ± 3.41	2.26 ± 5.65	2.35 ± 3.06	2.28 ± 6.56
18 days	2.28 ± 3.41	2.30 ± 5.6	2.48 ± 3.06	2.14 ± 6.56
28 days	2.39 ± 2.84	2.26 ± 5.71	2.38 ± 8.56	2.22 ± 8.08

Table 2 - Effects of treatments on optical density digital radiography images and biomechanical property

Histogram (pixel)	Sham PL	OVZ PL	OVZ 6CH	OVZ TM
6 days	300 ± 3.99 B	420 ± 5.78 C	300 ± 5.76 B	350 ± 10.1 AB
12 days	390 ± 4.87 BC	230 ± 6.89 A	320 ± 3.98 AB	390 ± 8.40 BC
18 days	400 ± 7.44 BC	250 ± 10.5 A	400 ± 6.43 BC	410 ± 7.25 BC
28 days	460 ± 0.27 C	260 ± 8.22 A	410 ± 9.87 BC	400 ± 9.25 BC
Biomechanical Test (N)	Sham PL	OVZ PL	OVZ 6CH	OVZ TM
6 day	4.5 ± 2.9 A	3.6 ± 2.86 AB	3.5 ± 2.95 AB	3.2 ± 4.75 AB
12 days	4.3 ± 3.54 A	2.8 ± 5.35 B	4.03 ± 4.36 AB	2.8 ± 5.16 B
18 days	4.9 ± 4.41 A	2.7 ± 4.46 B	4.82 ± 4.6 A	4.7 ± 5.4 A
28 days	5.8 ± 2.4 A	2.8 ± 2.91 B	6.01 ± 5.06 A	4.9 ± 4.08 A

The application of substances in homeopathic concentrations to animals offers the possibility to analyze the biological effect of the active principles, diluted and dynamized, according to homeopathic techniques on organisms, without considering the placebo effect [10]. Traditional Chinese medicine frequently use herbs for the treatment of many lesions, including bone repair [11].

A. montana is a medicinal plant used since ancient times to treat bruises and wounds in soft and hard tissues [12]. However, there are few scientific studies evaluating its effects on osteoporosis and repair of bone lesions in the presence of osteoporosis. Osteoporosis affects more elderly women, especially after menopause, so in this study, the experimental model with ovariectomized rats was chosen. A period of 45 days after Iovariectomy was sufficient for the development of bone abnormalities, suggestive of osteoporosis.

This experimental study aimed to evaluate the effect of treatment with *A. montana*, such as phytotherapeutic and homeopathic medicine, in the quality of bone repair in rats with osteoporosis, targeting the use of the plants as a medicine used in prophylaxis or treatment of osteoporosis and its complications, improving the quality of bone tissue. The ovariectomized group treated with *A. montana* 6CH had the best behavior in biomechanical test, while presented similar results in the others analysis. Oliosio et al. observed the positive effects of homeopathic use of *A. montana* and verified that it exhibited a remarkable tendency to increase bone morphogenetic protein (BMP2) which is required for the initiation of bone repair [8]. *A. montana* homeopathic remedy increases the energy metabolism of cells by protecting the mitochondria from oxidative damage caused by lipid peroxidation [13].

Measurement of length and diameter of the tibia in this study was a way to evaluate the effect of medicines on quality of bone tissue and possible interference with the mechanical properties of tibias.

Sham group showed the greater length of tibia at the end of the experiment, but this result was not statistically significant, and it can be hypothesized that osteoporosis has influence on the growth of long bones. The ovariectomized animals treated with TM arnica were those with shorter tibias at 18 and 28 days. This may introduce a reflection of toxicity of the treatment, since arnica has toxic active principles such as sesquiterpene helenalin [14].

Evaluation of bone regeneration by SEM showed good applicability regarding bone quality. Images acquired for the six days showed little bone formation in placebo-treated groups, and the OVZ PL group formed less bone than the SHAM PL. Both groups treated with *A. montana* formed more bone than placebo group and showed bone callous composed by trabecular bone occupying the entire defect area. It is necessary to highlight the animals treated with *A. montana* 6CH for 28 days, that showed good regeneration and bone quality when compared to other groups of the same time of treatment. Ovariectomized and treated animals with mother tincture showed good regeneration results by SEM analysis but, in the overall context, they were lower than the group treated with *A. montana* 6CH. The optical density of mother tincture group was better, however the biomechanical properties did not show good resistance as the group treated with Arnica 6CH. It is hypothesized that mother tincture of Arnica caused toxic effect, which may have affected bone growth.

The results of this study suggest new experiments in order to obtain a better understanding of the effectiveness of this medicinal plant for the treatment of osteoporosis and fractures in the presence of this disease. It is necessary to evaluate a longer time of treatment with homeopathic and phytotherapeutic Arnica and other mother tincture concentrations.

CONCLUSION

Based on the results obtained in the experimental conditions of this study, it can be

concluded that, although the treatment with homeopathic *A. montana* was more efficient than phytotherapeutic, in bone regeneration in rats with

osteoporosis, homeopathic or phytotherapeutic treatments acted positively in bone regeneration.

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Date submitted: 2016 Oct 28

Accept submission: 2017 Jan 31