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Research Article

Aqueous extract from Opuntia megapotamica fruit pulp promotes osteoblast activity

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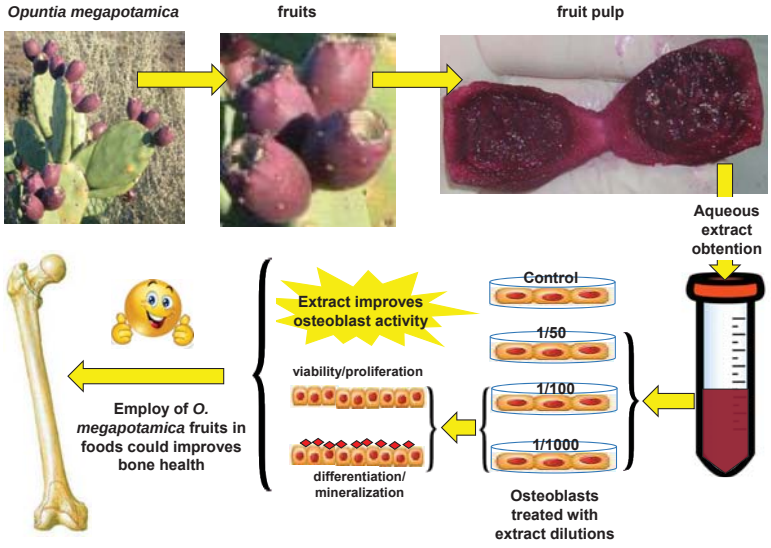
Abstract

Objetive: Osteoporosis and osteopenia are age-related chronic diseases with increased morbidity rates among postmenopausal women. Natural products investigation for prevention and treatment of these conditions is growing, due, in part, to the fact that they allow longer exposure times and minor secondary complications than synthetic drugs. *Opuntia* plants (Cactaceae) have been shown to possess a broad spectrum of medicinal properties. *Opuntia megapotamica* is an autochthonous species from Argentina but its action on bone metabolism has not yet been studied. The work aims is to evaluate the action of aqueous extracts of *O. megapotamica* fruits on the activity and function of osteoblasts *in vitro*.

Methods: Primary osteoblasts were obtained by digestion of the cranial bones of 3-5 day-old Wistar rats. Cells were treated with different dilutions of the aqueous extract (1/50-1/1000), then viability, proliferation, differentiation and mineralization were assessed at the corresponding time points.

Results: Dilution of extract 1/100 induced a slight but significant increase in cell viability with respect to control ($p < 0.05$) whereas the other conditions showed no changes. In addition, dilutions of extract 1/100 and 1/1000 significantly increased cell proliferation at 24 and 48 h with respect to the control ($p < 0.05$). Culture mineralization, showed a rising trend with respect to the control at 15 days of treatment with 1/1000 extract dilution.

Conclusions: The results show that the aqueous extract of *O. megapotamica* fruit increases the number and improves the biological functions of osteoblasts, suggesting its potential use as an alternative treatment for postmenopausal bone deficit.



Graphical abstract

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Introduction

Bone alterations and loss of bone mass have a great impact on world society, mainly in postmenopausal women. Older age, hormonal disorders, high impact diseases such as diabetes and celiac disease among other factors are frequently associated with deficits in bone formation [1-4]. Bone-forming cells, osteoblasts, originate by differentiation from mesenchymal stem cells, in response to a number of local and systemic factors [5]. The activity and function of these cells is crucial in the maintenance and regeneration of bone mass, the quality of the bone formed and the functioning of the skeletal system [6]. The main function of osteoblasts is to synthesize and secrete several proteins that integrate the organic extracellular matrix (such as cytokines, collagen and growth factors, among others) and convert the extracellular matrix into bone by mineralization [5]. Although there are numerous therapeutic approaches to stimulate bone tissue repair and regeneration, these are often insufficient. Therefore, efforts to find alternative therapies to stimulate osteogenesis should be increased [7,8].

The plant kingdom is a great reservoir, not fully explored, of biologically active compounds, not only as drugs but also as models from which synthetic analogs of therapeutic application can be obtained [9-11]. The World Health Organization (WHO) proposes to increase research aimed at examining the potential benefits of medicinal plants use in different pharmacological approaches for diseases treatments [12]. There is a growing interest in the study of natural products derived from plants for treatment of bone conditions [13-17]. This is, in part, because they offer the possibility of longer exposure times and fewer secondary complications compared to synthetic drugs [18,19]. In this sense, *in vitro* and *in vivo* studies showed that several compounds of vegetable origin such as flavonoids, steroids, anthraquinones, polyphenols, phenolic acid, terpenoids, coumarins and glycosides stimulate the proliferation and/or differentiation of osteoprogenitor cells [18,2-24].

The *Opuntia* (*Opuntia s.s.* Mill) are American continent native cacti that constitute the most numerous genus of the subfamily Opuntioideae, family Cactaceae, which is represented by around 190 species [25]. Traditionally, Latin America people have attributed to *Opuntia* spp. a broad spectrum of medicinal properties, among which its use for the treatment of diabetes stands out. On the other hand, its high content of polysaccharides, phenolic compounds and minerals such as calcium gives them high nutritional value [26-28]. It has been shown that numerous herbs have therapeutic actions on bone physiology [18,20,29-31]. However, the effects of *Opuntia* spp. on bone tissue still remain very little explored.

Various *Opuntia* species have shown a variety of biological effects: hypoglycemic and lipid-lowering [32-35], antioxidant [36-40], analgesic [41], anti-inflammatory [41], antiviral [42], hepatoprotective [43], antiulcerogenic [36], antiatherogenic [44] and antitumorigenic [45,46], actions, among others. The consumption of dehydrated cladodes of *Opuntia ficus indica*, source of bioavailable calcium, improves Bone Mineral Density (BMD) in adult women with low bone mass [47] and in weaned growing rats [48,49] On the other hand, the ethanolic extract of this same species prevents the decrease of bone mineral density induced by ovariectomy in rats [50]. The oral supply of polysaccharides isolated from cladodes, seeds and fruit pulp of *Opuntia humifusa* produces beneficial effects on the bone trabecular mass and on the structural properties of bone in ovariectomized female mice and rats with osteoporosis [51,52]. In *in vitro* studies, it was demonstrated that aqueous and ethanolic extracts of *O. humifusa* promote differentiation of murine osteoblasts and also decrease oxidative stress, suggesting an anabolic effect in bone [53].

Opuntia megapotamica is an autochthonous species from Argentina and, so far, has been very little studied. Complete pre-clinical studies conducted in Wistar rats, showed that flours of cladodes, fruit pulp and seeds of this *Opuntia* species are well tolerated when administered orally. In addition, these samples have a marked anti-hyperglycemic effect in rats with transient hyperglycemia, exert an important lipid-lowering action in diabetic rats and have positive effects on the pancreas structure and on liver function [54]. While these results may serve as a basis for future application of *O. megapotamica* as a natural therapeutic resource, there is no other background on its medicinal properties in the literature to date.

In the present work, it was hypothesized that fruit pulp of *O. megapotamica* would improve osteogenesis. In order to address this, it was studied the effect of aqueous extracts from *O. megapotamica* fruits pulp on the activity and function of neonatal rat calvarial cells.

Materials and methods

Materials

Alizarin Red, Ascorbic Acid, β-glycerophosphate and a-MEM (1.36 mM Ca^{2+}; $CaCl_2.2H_2O$ 0.2g/L) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Fetal bovine serum (FBS) was from Natocor (Córdoba, Argentina). Alkaline phosphatase (ALP) activity kit was donated by Wiener Lab. Rosario, Argentina. Neutral Red dye was donated by Paula Messina, PhD (Chemistry Department of Universidad Nacional del Sur, Bahía Blanca, Argentina). All other reagents used were of analytical grade.

Vegetable material collection

A cactus population from *Opuntia* (Mill.) genus that grows spontaneously in Coronel Rosales party Buenos Aires province was used, an image of it is shown in Figure 1. This material has been the object of recent systematic studies and belongs from a variety not yet described of the *Opuntia megapotamica* complex (Villamil, com. Pers). A reference specimen (Villamil 8829) is deposited in the Herbarium of Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur (BBB), Bahía Blanca, Argentina. Material collection, post-harvest processing and storage were carried out taking into account the general guidelines applicable to succulent plants and vegetable raw materials in the phytotherapeutic product industry [55,56]. The fruits were collected from different adult

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Figure 1: *Opuntia megapotamica* specie. Wild specimen of *O. megapotamica* growing naturally in the vicinity of the Bahía Blanca town, south of the Buenos Aires province, Argentina. **A)** Cladodes and ripe fruits seen in more detail. **B)** A plant in the state of flowering.

plants chosen at random and the turgid fruits were selected based on their morphology parameters such as external color, size, surface, texture, odor, taste, and thickness (mature, about 7 cm long and 4 cm wide that were still attached to the plants). In the first stage of post-harvest processing fruit parts with deterioration, stains and/or with signs of attack by pathogens were discarded. After peeling the fruits, they were sectioned in half and the pulp was separated from the seeds. Next, fruit pulp was frozen at -40ºC for 12 h and then lyophilized. Once dry, the material was stored, under vacuum and protected from light, at -20ºC until the moment of its use. We worked with the dehydrated plant material because the drying process interrupts degradation caused by enzymes and prevents development of microorganisms and oxidation and hydrolysis reactions.

Preparation of extracts from plant material

Approximately 1 g of lyophilized material was extracted with 10 ml of double-distilled water under stirring for 2 h, at 25°C. After maceration, the aqueous extract was filtered with 0.22 µm pore diameter filters and frozen at -20°C until further use.

Cell isolation

Calvarial cells were obtained from 3-5-day-old neonatal Wistar rats. Animals were sacrificed by fast decapitation. All procedures were carried out in conformity with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) under protocol nº 012/2014 of Institutional Animal Care and Use Committee (CICUAE) of Universidad Nacional del Sur, Argentina. The head of each animal was immersed in a series of three beakers containing 70% ethanol (3 times in each beaker, for 1-2 seconds) and the calvaria was extracted after removing the skin and skull tissue. After removing all soft tissue debris, the cranial bones were incubated in phosphate buffer saline (PBS) containing 4 mM EDTA at 37°C for two 10-min periods and the supernatants were discarded. Subsequently, the cranial bones were rinsed in PBS and subjected to enzymatic digestion, by incubation in PBS with collagenase (200 U / ml) for 15 min, at 37ºC. The solution containing the cells released in this first digestion was discarded. A second enzymatic digestion was then performed, incubating the bones in PBS containing collagenase (200U / ml) for 15 min, at 37 ° C. The solution containing the cells released in this second digestion was collected and subjected to centrifugation at 1200 rpm for 10 min. The supernatant was then discarded and the cell pellet was resuspended in α-MEM supplemented with 15% FBS to stop the enzymatic action. After this, the bones underwent two other enzymatic digestions like the previous one and the collected cells were pooled with those obtained previously. Then, cells were cultured at 37°C in α-MEM supplemented with 15% FBS, 1% penicillin and streptomycin under humidified air (5.5% CO_2). After 24 h, the medium was replaced by α-MEM supplemented with 10% FBS, 1% penicillin and streptomycin and the cells were cultured until ~80 % of confluence (2-3 days). Cells were frozen in liquid nitrogen until their use.

Cell culture and treatment

Cells were thawed and seeded into 10 cm diameter glass Petri dishes and allowed to grow to 80% confluence. Then, cells were passed at a density of 10×10^3 cells/cm^2 and cultured for 3-4 days in α-MEM supplemented with 10% FBS, in a humidified atmosphere (5.5% CO_2) at 37°C. For each experiment, the cells underwent two passages, one when they were obtained before being frozen, and the other when they were counted and seeded to the various experimental conditions tested. When cells reached 80% confluence, they were starved in 1 % FBS medium for 7-16 h before starting treatment. This procedure allows to arrest the cell cycle and thus synchronize the culture, avoiding the use of dexamethasone, which can interfere with osteogenic differentiation. Treatments were performed by replacing the medium by treatment medium (α-MEM supplemented with 1% FBS, containing the indicated amounts of extract or the vehicle/water pH:7 used in control conditions). When experiment where performed in osteogenic conditions, 2 mM β-glycerophosphate and 50 µg/ml ascorbic acid where incorporated to treatment medium. The control or treatment medium was renewed every 2-3 days.

Cell viability assay

Determination of cell viability was carried out using Neutral Red staining. After treatments, cells were washed with PBS 1X and stained with Neutral red for 2-3 h, at 37 ºC. Dye excess was removed with PBS 1X and photograph of the cells were taken. Finally, the dye incorporated to cells was extracted with remover solution (50% ethanol 96%, 49% deionized water, 1% glacial acetic acid) and quantified, at 540 nm, in a spectrophotometer with plate reader (Sinergy HT plate reader, BioTek).

Cell proliferation assay

Determination of cell proliferation was carried out using Crystal Violet staining. After treatments, the cells were washed with PBS 1X, and fixed with pure methanol, for 10 minutes at 37 ºC. The alcohol was then removed with distilled water and staining was started with Crystal Violet dye, which was performed for 30 minutes at room temperature. Excess dye was removed with distilled water. Finally, the dye was extracted, for 30 minutes at room temperature, with Triton 0,2%. Spectrophotometric quantification of the extracts was carried out in plate reader, at a wavelength of 590 nm.

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Alkaline phosphatase (ALP) activity

The ALP activity of cell was colorimetrically determined using a commercially available kit (Wiener Lab., Rosario, Argentina); this assay uses sodium phenylphosphate as a substrate; ALP in the presence of methyl propanol amine (pH 10) releases phenol. The phenol released is combined with a color generating reagent solution of 4-amino-antipyrine and ferrocyanide and quantified at 520 nm. The ALP activity was measured after the indicated cell treatments. Briefly, cell layers were washed with PBS and then cells were incubated with 50 µl/well of PBS containing 0.2% Triton for 10 min at 37ºC. Follow 250 µl of substrate was added to each sample and it was incubated for 10 min (37ºC) before the addition of 1.25 ml of color reagent and quantification as indicated above. A blank (B) and standard (S) (200 IU/L phenol) were also processed. Optical density of the samples (D) was measured and ALP activity was calculated as follows: ALP (IU/L) = 200IU/Lx (D-B)/(S-B).

Alizarin red staining

Calvarial osteoblasts seeded in 48-well plates were cultured and treated as above for 15 days. The cells were then fixed with 2% glutaraldehyde in PBS (pH 7.4) at room temperature for 10 min, washed three times with PBS, and then incubated with 2 % Alizarin Red (pH 4.2) for 30 min at 37°C. The cells were washed thoroughly with deionized water. Stained cells were observed under inverted microscope (Carl Zeiss, USA) and photomicrographs captured using image analyzer (Carl Zeiss, USA). Following staining technique, evaluation of mineral distribution and inspection of fine structures by microscopy was performed. Then for quantification of Alizarin Red staining, the samples were incubated with 0.5 ml of 500 µM NaOH during 5 min and O.D. (Absorbance) was measured in the plate reader spectrophotometer at 548 nm.

Statistical analysis

InfoStat software was employed. Variance Analysis (ANOVA) was used to determine differences between the average values of several statistical populations. The mean values were compared by the *post hoc* test of multiple comparisons by Bonferroni. The letters (a-b-c) indicate significant differences ($p \leq 0,05$) between the groups. The represented values correspond to the average ± standard deviation (SD). The total number of samples is indicated in the legend of each figure or table.

Results

Effect of aqueous extract from O. megapotamica fruits on cell viability of rat calvarial cell cultures

In order to study the effect of extract from *O. megapotamica* fruits pulp on cell viability of rat calvarial cultures, we used the Neutral Red staining. It is extensively employed as a convenient and rapid assay for measuring cell viability. It is a well-known quantitative colorimetric method based on the uptake of the weakly cationic dye Neutral Red which enters into the cell by diffusion through the cell membrane. Then, the dye accumulates in the lysosomes of living cells [57]. The cells were treated with different dilutions (1/50, 1/100, 1/1000) of aqueous extract for a period of 7 days after which viability assay was performed as indicated in Materials and Methods. In none of the conditions treated with the different dilutions of the extract decreased cell viability was observed. In contrast, 1/100 extract dilution caused a statistically significant increase on cell viability with respect to the control (Figure 2). This data suggest that aqueous extract from *O. megapotamica* fruit pulp does not elicit negative effects on the viability of rat calvarial cells.

Effect of extract from O. megapotamica fruits on cell proliferation of rat calvarial cell cultures

To assess the effect of aqueous extract from *O. megapotamica* fruits pulp on cell proliferation, studies were performed by staining with crystal violet. This is a fast, efficient, accurate, sensitive and reliable method to detect changes in both adhesion and *in vitro* cell proliferation as well as for cytotoxicity studies. The dye interacts with proteins and DNA. Thus, color intensity is directly proportional to the cell biomass and can be easily measured by spectrophotometry. Therefore, increases in the cell number of a sample, result in a quantitative increase in staining. Similarly, when cells lose their adhesion, they detach from cell population, resulting in decreased staining [58]. Cells

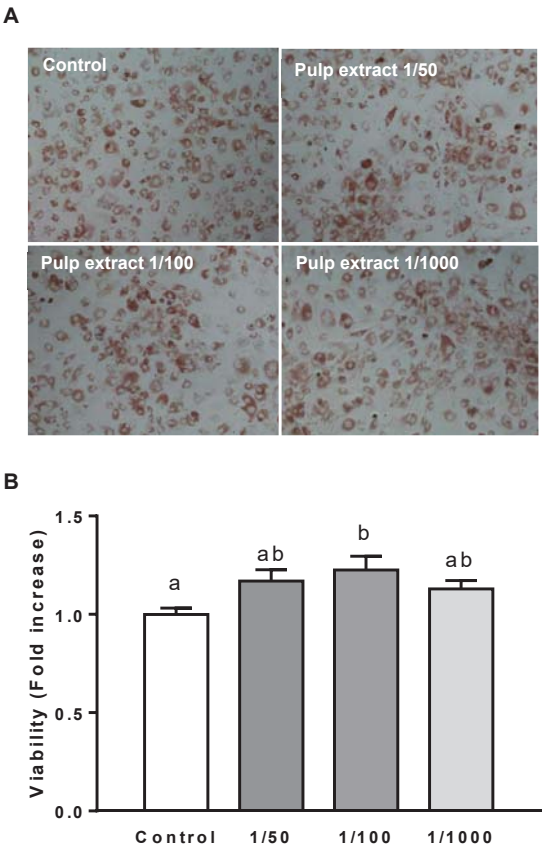

Figure 2: Effect of *O. megapotamica* aqueous pulp extract on cell viability of neonatal rat calvarial primary cultures. Cells were treated with vehicle (control) or the indicated dilutions of *O. megapotamica* fruit pulp extract for 7 days. Then, cell viability was determined by neutral red staining as indicated in Materials and Methods. A) Representative images of cells stained with neutral red are shown; B) Data represent the mean ± SD from at least three independent experiments, each performed with 4 wells per condition. Different letters indicate statistical differences (ANOVA -Bonferroni $p < 0.05$).

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were treated with different dilutions (1/50, 1/100, 1/1000) of aqueous extract for 24, 48 and 72 h and then where stained with crystal violet as indicated in Materials and Methods. It was only detected changes in those conditions treated with the 1/100 and 1/1000 extract dilutions, which showed a significant increase in crystal violet staining at 24 and 48 h respect control. This results suggest that the extract does not exhibit cytotoxicity or affect cell adhesion. In addition, it stimulates cellular proliferation at 1/100 and 1/1000 dilutions (Figure 3).

Effect of extract from O. megapotamica fruits on ALP activity in rat calvarial cell cultures

Alkaline phosphatase (ALP) activity is considered an early-stage differentiation marker of the osteoblast phenotype. In rat calvarial cell cultures, it has been shown that ALP increases in the first weeks (4-10 days) of culture in osteogenic medium [59-61]. Thus, in order to evaluate the effect of extracts from *O. megapotamica* fruits pulp on rat calvarial cell differentiation into osteoblast, ALP activity was measured in cells treated with different dilutions (1/50, 1/100, 1/1000) of aqueous extract for 7 days in osteogenic medium, as indicated in Materials and Methods. At the time studied, vegetal extracts didn´t stimulate enzyme activity and even a slight decrease was found in those cells exposed to dilution 1/50 (Figure 4).

Effect of extract from O. megapotamica fruits pulp on mineralization of rat calvarial cell cultures

Mineralization is considered a functional *in vitro* endpoint reflecting advanced osteoblastic cell differentiation [59]. Rat calvarial cells reach a mature osteoblast stage after long incubation times (>15 day) in osteogenic medium. Mineralization nodules can be visualized around day 15-20, or even later in such conditions [59-61]. In order to study if extract from *O. megapotamica* fruits pulp elicit any action in rat calvarial cell culture mineralization, the effect of cell treatment with aqueous extract on extracellular matrix mineralization after long incubation times was assessed. This was performed

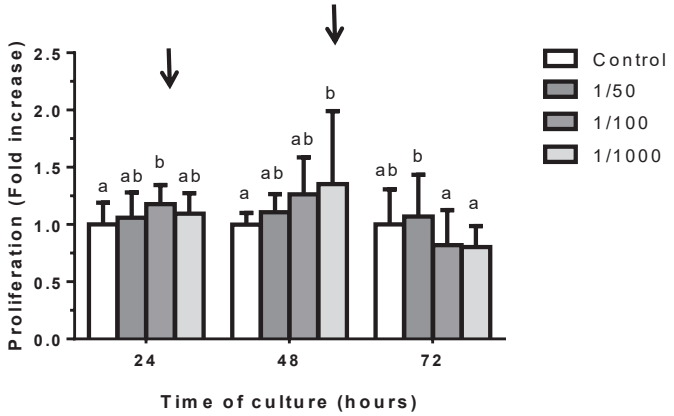

Figure 3: Effect of *O. megapotamica* aqueous pulp extract on cell proliferation of neonatal rat calvarial primary cultures. Cells were treated with vehicle (control) or the indicated dilutions of *O. megapotamica* extract for 24, 48, and 72 h. Then cell proliferation was determined as indicated in Materials and Methods. Data represent the mean ± SD from at least three independent experiments, each performed with 4 wells per condition. Different letters indicate statistical differences (ANOVA -Fisher $p < 0.05$).

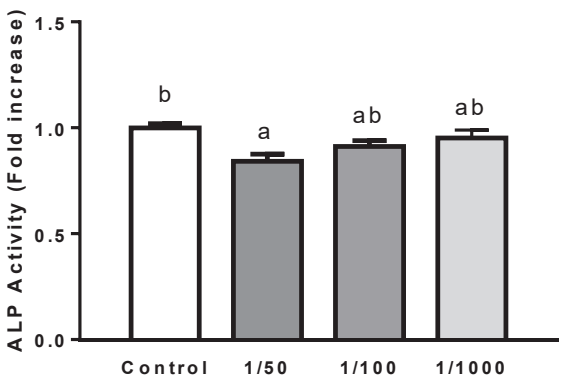

Figure 4: Effect of *O. megapotamica* aqueous pulp extract on Alkaline Phosphatase (ALP) Activity of neonatal rat calvarial primary cultures. Cells were treated with vehicle (control) or the indicated dilutions of *O. megapotamica* extract for 7 days in osteogenic medium. Then, quantification of ALP activity was determined as indicated in Materials and Methods. Data represent the mean ± SD of at least three independent experiments, each performed with 4 wells per condition. Different letters indicate statistical differences (ANOVA -Bonferroni $p < 0.05$).

by determining the calcium deposits on cell cultures using the Alizarin Red organic dye, as described in Materials and Methods. Cells were treated with different dilutions of aqueous extract (1/50, 1/100, 1/1000) in osteogenic medium for 15 days. Cells grown in the presence of 1/100 extract dilution showed a tendency to reduce calcium deposition, respect to control. While, cell treatment with 1/1000 extract dilution showed a tendency to increase mineralization with respect to control (Figure 5). This result suggests that long time exposition of cells to 1/100 dilution does not favor osteoblasts mineralization whereas the most diluted extract does it.

Discussion

In the present study, we show for the first time an evaluation of the osteogenic potential of extracts from *O. megapotamica* fruit pulp using neonatal rat calvarial cells cultures. This cultures are a heterogeneous cell population majorly containing mesenchymal stem cells, committed osteoprogenitor cells, preosteoblasts and osteoblasts. Under appropriate conditions, these cell cultures can differentiate into osteoblasts, condrocytes or adipocytes as it has been described [62,63]. Under osteogenic conditions, the osteoblasts precursor cells have an osteoblast development pattern similar to *in vivo* bone formation. Thus, these cultures are a well-accepted model of osteogenesis *in vitro*. During the culture proliferative phase, the cells undergo DNA synthesis and cell division, resulting in a rapid increase in cell number until confluence. Then, proliferation is arrested, and begin to sequentially increase the mature osteoblast characteristics, such as alkaline phosphatase (ALP) production, conversion of procollagen to collagen, and deposition of extracellular matrix on the substrate, which is subsequently mineralized [59-61]. The conducing mechanism of osteogenesis has not been fully clarified. Although various osteogenic formulations are known, as well as the importance of each one of their constituents for induction of *in vitro* osteogenic differentiation, this has not yet been fully optimized, generating wide interest in discovering new osteogenic active principles [64].

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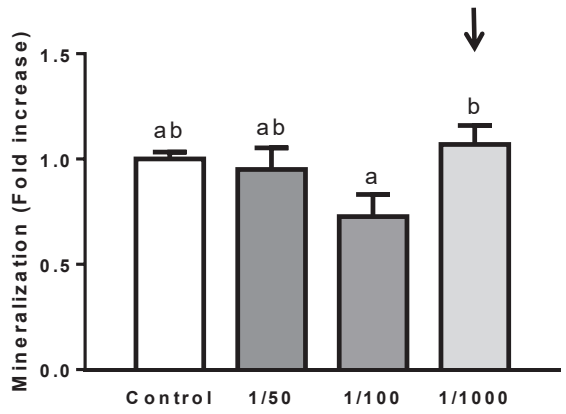

Figure 5: Effect of *O. megapotamica* aqueous pulp extract on mineralization of neonatal rat calvarial primary cultures. Cells were treated with vehicle (control) or the indicated dilutions of *O. megapotamica* extract for 15 days in osteogenic medium. Then, cultures mineralization was determined by Alizarin red staining as indicated in Materials and methods. Data represent the mean ± SD of at least three independent experiments, each performed with 4 wells per condition. Different letters indicate statistical differences (ANOVA - Bonferroni $p < 0.05$).

One of the criteria used to identify active ingredients of compounds at the bone level comprises the lack of toxicity and the promotion of osteoblast activity. In this sense, our results show that aqueous extracts from *O. megapotamica* fruit pulp are not toxic for osteoblast, because no negative effect on cell viability or adherence were observed at any of the dilutions used here. We employed a considerably long time (7 days), during which no change in cell morphology in the different conditions were observed respect to control (not shown). Thus, suggesting that the extract do not elicit negative effects on cell physiology in the short nor in the long time. Moreover, a statistically significant augment on cell viability was observed at dilution 1/100. This could be due to an increase in cell number since the extract induced a significant stimulation of cell proliferation at dilution 1/100 and 1/1000. The stimulation of cell proliferation is also a beneficial feature of the extracts because of its potential application for the treatment of bone conditions such as osteoporosis or fractures, by promoting an increase in the size of the active cell population. In agreement with our results, extracts of fruits of other *Opuntia* species have been shown to stimulate cell proliferation, such as *O. Humifusa* on MC3T3-E1 osteoblastic cells [53], and *O. ficus indica* on human fibroblasts and keratinocytes [65]. Unlike, antiproliferative effects for extracts of fruits of *O. humifusa* have been evidenced in human cervical carcinoma cells [45], and for *O. ficus indica* in human colorectal carcinoma (Caco-2) cells [66]. Of relevance, we have not found studies on the effect of extracts of *O. megapotamica* on the growth of tumor cells, which we visualize interesting for future investigations.

Another desirable effect of active principles at the bone level is promoting cell differentiation, since increases in the population of mature osteoblasts would favor bone formation. ALP is a protein involved in bone metabolism and one of the marker enzymes of mature osteoblasts, which plays an important role during calcification *in vitro [67]*. Its level commonly increases in the early period of osteoblastic differentiation and decreases in the mineralized period [68]. Our results show that the extracts, at the dilutions used here, do not significantly affect ALP activity. In discrepancy with our results, i*n vitro* studies demonstrated that aqueous and ethanolic extracts of *Opuntia humifusa* promote differentiation of murine osteoblasts, suggesting an anabolic effect in bone [53].

Bone mineralization occurs by a set of physicochemical and biochemical processes that facilitate the deposition of hydroxyapatite crystals both along the collagen fibrils in the extracellular matrix, as well as within the lumen of the matrix vesicles derived from osteoblasts [69,70]. In our study we did not detect significant change in culture mineralization by treatment with *O. megapotamica* fruit extract. However, the 1/1000 dilution showed a tendency to increase mineralization, suggesting that the extract can elicit a positive anabolic effect on bone. In agreement with this, studies in Mexican adult women have shown that the intake of dehydrated cactus of *O. ficus indica* improves bone mineral density and calciuria [47]. Also, studies on bone metabolism in rats suggests that supplementation with *O. humifusa* would have a positive effect on bone formation and the achievement of peak bone mass in the growth period by increasing the levels of the osteogenic marker osteocalcin [48]. The active compounds of *O. megapotamica* fruit extracts and the mechanisms of action involved on osteoblast physiology are not known and they would be an interesting goal for future research.

Opuntia megapotamica is an autochthonous species from Argentina. Complete pre-clinical studies conducted in Wistar rats, showed that flours of cladodes, fruit pulp and seeds of this cactaceae are well tolerated when administered orally [54]. These results, together with the present study, may serve as a basis for future chemical analysis of *O. megapotamica* extracts and for investigations on application of *O. megapotamica* as a natural therapeutic resource for treatment of metabolic bone diseases. It is relevant to consider the ecological, economic and social advantages that the medical and/or nutraceutical use of *O. megapotamica* could have, since it is a rustic plant, easy to spread, manipulate and process and that grows in arid and semi-arid zones where other crops don´t develop naturally.

In addition, it is important to note that plant extracts as other naturally available materials such as vitamins, carbohydrates and biodegradable polymers, among others, can act as solvents, reducing agents and/or stabilizers in the synthesis of nanoparticles for development of bio-materials applicable in medicine [71-73]. In this sense, we consider that *O. megapotamica* extracts are of potential application in the biological or chemical synthesis of hydroxyapatite nanoparticles for use in certain medicine branches such as orthopedics, orthodontics and plastic surgery. Related to this, there is a great interest in replacing hazardous materials with green chemicals in order to protect human health and the environment [74,75].

Potential clinical value

These findings indicate that *O. megapotamica* is a target of interest for the search for new osteogenic drugs and also

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presents potential application as a complementary therapy to current treatments for postmenopausal osteoporosis.

Conclusion

In conclusion, our results suggest that extracts of *O. megapotamica* fruit pulp have a beneficial effect on the activity and function of osteoblasts. This together with the ecological, economic and social advantages of *Opuntia* spp. cultivation will be a starting point to promote the implementation of clinical studies with fruit extracts of *O. megapotamica*, as a potential bone anabolic agent for the treatment of bone deficiency associated diseases, including postmenopausal osteoporosis.

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