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Stimulating effects of hydro-ethanolic and acetonic extracts of *Phaseolus vulgaris* (Fabaceae) on proliferation and differentiation of osteoblastic cells *in vitro*

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Abstract

Extracts from *Phaseolus vulgaris* (Fabaceae) were studied for their potential effects on the metabolism of osteoblastic cells. Results indicate that the hydro-ethanolic and acetonic extract stimulates the proliferation of ROS cells. The stimulative effects induced by hydro-ethanolic extract persist even in the presence of an inhibitor of estrogenic receptor such as Tamoxifen. The addition of Tamoxifen in the culture medium inhibits the effects induced by acetonic extract of *Phaseolus vulgaris* for 10^{-6} and 10^{-5} g/ml doses of the extract. For higher doses of extract such as 10^{-4} and 10^{-3} g/ml, the stimulative effects of acetonic extract is not affected by Tamoxifen. When the Tamoxifen is added to the media before extracts, effects induced by plant extracts persist only at 10^{-3} g/ml. The effects disappear at 10^{-6}, 10^{-5} and 10^{-4} g/ml. The hydro-ethanolic extract stimulates the differentiation of osteoblastic ROS cells whereas the acetonic extract has no such effect. The effects induced by the hydro-ethanolic extract disappear in the presence of Tamoxifen. Our results indicate that extracts of *Phaseolus vulgaris* stimulate both the proliferation and the differentiation of osteoblastic cells. These actions could be due, among other reasons, to phyto-estrogens in the extract acting, at least partially, on the estrogens receptors. These molecules may also act through mechanisms where estrogen receptors are not involved. Our results suggest the presence of non-estrogenic substances and/or acting through mechanisms different from those of the estrogen receptors.

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Introduction

The advances and innovations of modern medicine in the last decades have significantly increased life expectancy among humans. However, elderly people health is affected by disorders resulting from bone degeneration such as osteoporosis. This has become a serious health issue and an important part of health budget in many countries.

As the search for an efficient cure has been difficult throughout the years, the use of medicinal plants is becoming a serious alternative (Ososki and Kennely, 2003). There are several mechanisms of action for substances efficient against bone degeneration but one can keep in mind that phyto-estrogens, stimulate the proliferation and differentiation of osteoblasts, and the synthesis of the bone matrix. Phyto-estrogens are efficient against the bone degeneration in ovariectomized rat and postmenopausal women (Arjmandi *et al.*, 1996; Draper *et al.*, 1997; Morabito *et al.*, 2002; Shirke *et al.*, 2009). Conversely, phyto-estrogens inhibit osteoclasts (Rassi *et al.*, 2002). Phyto-estrogens, such as anthocyanins, can also play a role of antioxidant (Kong, 2003).

Soy (*Glycine max*) is one of the sources of these phyto-estrogens. Several studies demonstrated the biomedical properties of the phyto-estrogens of soy seeds (Ososki and Kennely, 2003; Chen and Anderson, 2002; Om and Shim, 2007). Based on the chemical composition of *Phaseolus vulgaris* seeds, we suggest that this plant might be efficient against bone diseases. Indeed, it has been demonstrated that extracts of *Phaseolus vulgaris* seeds contain many phyto-estrogens including estrone, estriols and 17 - estradiols (Kopcewicz, 1971; Janeczko and Skoczowski, 2005), daidzein, genistein and secoisolariciresinol (Mazur, 1998; Boue *et al.*, 2003; Shirke *et al.*, 2009). Moreover, Paris and Venkateswaran (2003) showed that 100g of seeds of *Phaseolus vulgaris* contain up to 50 mg of phyto-estrogen.

Therefore, the objective of this work is to analyze the stimulative effects of extracts *Phaseolus vulgaris* on the proliferation and the differentiation of osteoblastic cells. The motivation of these studies is to know if the extracts of *Phaseolus vulgaris* beans widely used in human alimentation could be useful to cure bone diseases such as osteoporosis.

Materials and methods

Preparation of the plant extracts

Plant extracts were prepared as described by previous authors (Benié and Thieulant, 2003, Ye *et al*, 2003). In brief, dried plants (broad beans) were pulverized with a mortar. Twenty grams of powder were mixed with 500 ml solvent. The mixture was left for 4 hours at room temperature and then subjected to magnetic stirring for 30 minutes and finally filtered on Whatman paper. The filtrate was evaporated under low pressure at a temperature varying between 40 and 70°C depending on the type of solvent, ethanol (80%) or acetone. The acetone extract was applied on a silica gel column and fractionated by successive elutions with hexane, hexane-ethylacetate (10/1, v/v), hexane-ethylacetate (5/1), ethylacetate and acetone.

The fractions were collected and evaporated under low pressure at 43°C in the dark.

The different extracts and fractions were dissolved in PBS and tested for biological activity on bone cells.

Cell culture

Experiments were performed on rat osteosarcoma cells (ROS 17/2.8). Stock cultures of ROS 17/2.8 were maintained in MEM supplemented with 10% FCS (Cambrex BioSciences, Verviers, Belgium) [Egrise, 1992; Ogata *et al.*, 2012].

Cells were cultured in 37°C, in a humidified atmosphere of 95% air and 5% CO_2 with antibiotics [1% volume fraction penicillin-streptomycin-neomycin solution (Sigma chemical, St Louis MO, USA)].

Cell proliferation

Cell proliferation was estimated by measuring the incorporation of ^{3}H-thymidine that is an estimator of DNA synthesis (Egrise *et al,* 1990; Choi *et al,* 2001; Duque and Rakic, 2011).

The osteoblastic cells were plated at a density of 5.10^3 cells/well in 96-well culture plates in standard medium. 48 hours later, the medium was replaced by MEM supplemented with 1 mg/ml Albumax (In Vitrogen, Gent, Belgium) and different concentrations of plant extract or vehicle (PBS). In some of the experiments, tamoxifen (Sigma-Aldrich, Bornem, Belgium) was added at 10^{-6} g/ml, 30 minutes either before or after the addition of plant extracts. The cells were pulsed with 1 µCi of ^{3}H-thymidine (Moravek Biochemicals, Isobio, Fleurus, Belgium) for the last four hours of the 48 hours incubation time. At the end of the experiment, the cell layer was washed twice with ice-cold 10% trichloracetic acid and once with an ethanol/ether mixture. The trichloracetic acid insoluble material was dissolved in 0.2 N NaOH and ^{3}H-thymidine incorporation in the DNA was counted by liquid spectrometry. The results were expressed in cpm/ well.

Phenotypic characterization of the cells : alkaline phosphatase activity measurement

The osteoblastic cells were plated at a density of 2.10^4 cells / well in 24-well culture plates in standard medium. 48 hours later, the medium was replaced with MEM supplemented with 1 mg/ml Albumax (Invitrogen, Gent, Belgium) and with different concentrations of plant extract or vehicle (PBS). In some of the experiments, tamoxifen (Sigma-Aldrich, Bornem, Belgium) was added at 10^{-6} g/ml, 30 minutes either before or after the addition of plant extracts. At the end of the 48h incubation period, the culture medium was removed, the cell layer was washed with PBS and then scraped off with a rubber scraper and sonicated in distilled water. After centrifugation, the supernatants were used for the determination of alkaline phosphatase activity (APA) and protein content (Coomassie brilliant blue G-250 dye binding method). The alkaline phosphatase activity was measured by spectrophotometry using p-nitrophenylphosphate (Sigma –Aldrich) as substrate. APA was expressed in mU / mg protein.

Statistical analysis

Results were expressed as mean ± SEM of triplicates. Statistical analysis was performed using two-tailed Student's t test.

Results

Effect of hydro-ethanolic and acetonic extracts of Phaseolus vulgaris on the proliferation of bone cells

Hydro-ethanolic extracts of *Phaseolus vulgaris* (5.10^{-6} to 10^{-4} g/ml) increased dose-dependently ^{3}H-thymidine incorporation in ROS 17/2.8 cells (Fig. 1A). The highest increase (166 %) was observed at 5.10^{-5} g/ml.

The acetonic extracts markedly increased ^{3}H-thymidine incorporation in ROS 17/2.8 between 10^{-6} and 10^{-4} g/ml. The highest increase (379 %) was observed at 10^{-4} g/ml (Fig. 1B).

All fractions (hexane, hexane/ethylacetate 10/1, hexane/ethylacetate 5/1, ethylacetate and acetone) recovered from the chromatography on a silica gel column of the acetonic extracts increased significantly (from 20 to 50 %) the proliferation of ROS 17 / 2.8 (Fig. 2).

Modulation of the effect of hydro-ethanolic and acetonic extracts on the proliferation of bone cell by tamoxifen

The addition of 10^{-6} g/ml tamoxifen in the culture medium 30 minutes before or after the addition of the hydro-ethanolic extracts did not modify their stimulatory effects on the proliferation of ROS 17/2.8 cells (Fig 3).

The addition of 10^{-6} g/ml tamoxifen in the culture medium 30 minutes before or after the addition of the acetonic extracts completely neutralized their stimulatory effect on ROS cells proliferation when the extracts were added at 10^{-6} or 10^{-5} g/ml, and considerably reduced the effect induced by the acetonic extract at 10^{-4} g/ml (Fig 4).

Tamoxifen showed no effect per se on ROS 17/2.8 proliferation.

Effect of hydro-ethanolic extracts on alkaline phosphatase levels of bone cells.

All concentrations tested of the hydro-ethanolic extracts increased the levels of alkaline phosphatase in ROS 17/2.8 (Fig 5). Acetonic extracts demonstrated no effect on alkaline phosphatase expression (data not shown).

Modulation by tamoxifen of the effect of hydro-ethanolic extracts on alkaline phosphatase levels in bone cells

The addition of 10^{-6} g / ml tamoxifen 30 minutes before or after the hydro-ethanolic extracts abolished their stimulatory effects on alkaline phosphatase levels in ROS 17/2.8 (Fig. 6).

Discussion

We tested the effects of *Phaseolus vulgaris* on the proliferation of osteoblastic cells. Results showed that hydro-ethanolic and acetonic extract of *Phaseolus vulgaris* stimulate the proliferation of the ROS osteoblastic cells.

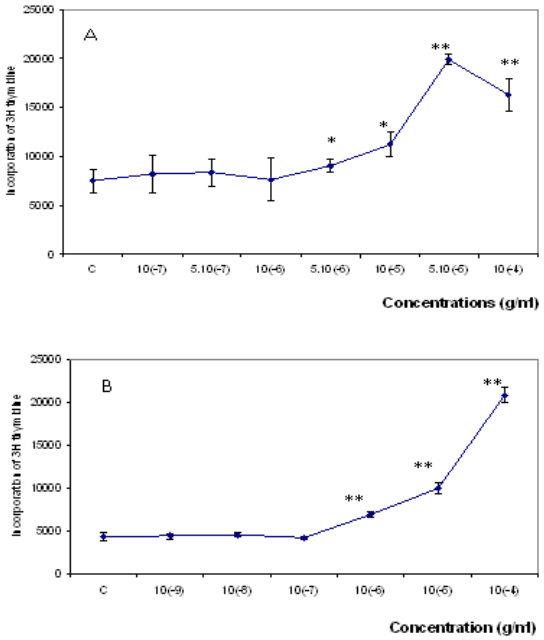

Fig. 1. Effect of *Phaseolus vulgaris* extracts on the proliferation of osteoblast-like cells**.**

Osteoblast-like cells were incubated for 48 hours in MEM supplemented with 1 mg/ml Albumax supplemented with different concentrations of plant extracts or vehicle. The cells were pulsed with1 µCi ^{3}H-thymidine for the last 4 hours. Results (mean of triplicates ± SEM) indicate the number of ^{3}H-thymidine cpm counted per well.

Student's t test : difference between cells treated with vehicle , * $p < 0.05$; ** $p < 0.01$.

Fig 1A : effect of hydro-ethanolic extracts on ROS 17/2.8 cells.

Fig 1B : effect of acetonic extracts on ROS 17/2.8 cells.

Our results are similar to those obtained by many authors on different species. Jeong *et al.* (2005) reported the stimulative effects of *Drynariae rhizoma* extracts on the proliferation of osteoblastic MC3T3-E1 cells. Choi *et al.* (2001) and Oh *et al.* (2003) showed that ethanolic extract of soy beans (*Glycine max*) and extract of *Rehmannia glutinosa* Libosch have a stimulative action on the cell proliferation, respectively.

The consumption of dry common beans (*Phaseolus vulgaris*) has been associated with a decrease risk for a wide variety of chronic and degenerative diseases such as cancer, obesity, diabetes and cardiovascular diseases (Reynoso-Camacho *et al.,* 2006). Beans are considered as a good source of high protein content, complex carbohydrates, dietary fiber and some vitamins and minerals. In addition to these nutritional components, common beans are rich in a variety of several phytochemicals with potential health benefits such as polyphenol compounds, fiber, lectins and trypsin inhibitors, among others (Reynoso-Camacho *et al.,* 2006).

Other authors reported the presence of phyto-estrogens in extracts of *Phaseolus vulgaris* [estrone, estriol, 17α-estradiol (Kopcewicz, 1971; Janecko and Skoczowski, 2005); Daidzein, Genistein, Secoisolariciresinol (Mazur, 1998, Boue *et al.*, 2003)]. Moreover, similar stimulative effects of by phyto-estrogens on cell proliferation have been reported by many authors. Choi (2005) demonstrated that the glabridine increases osteoblastic functions of MC3T3-E1 cells. Daidzein, genistein and resveratrol (Cho *et*

al., 2002; Mizutani *et al.*, 1998) have some stimulative effects on osteoblasts cells.

We argue that the proliferative effects of *Phaseolus vulgaris* extracts on the osteoblastic cells are due to the phyto-estrogens found in these extracts (Lee *et al.*, 2004). We prepared different fractions (hexane, hexane-ethylacetate (10/1), hexane-ethylacetate (5/1),ethylacetate and acetone) from acetonic extracts because these extracts seem more active on the proliferation than the hydro-ethanolic extracts.

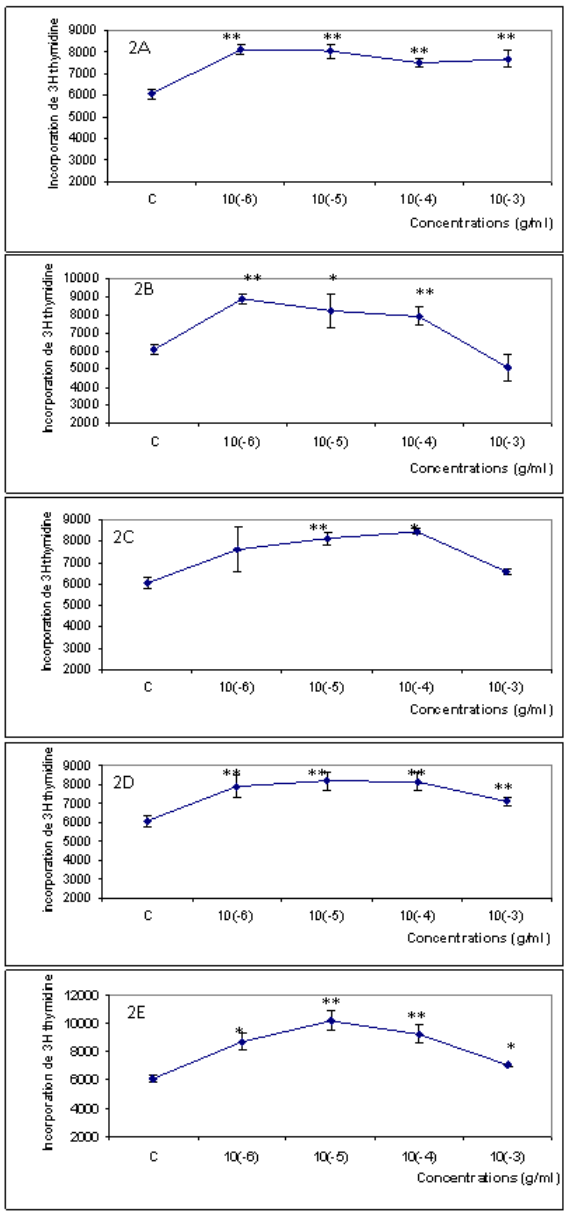

Fig. 2. Effect on ROS 17/2.8 proliferation of different fractions of acetonic extracts.

Osteoblast-like cells were incubated for 48 hours in MEM supplemented with 1 mg/ml Albumax supplemented with different concentrations of the fractions (hexane, hexane/ethylacetate 10/1, hexane/ethylacetate 5/1, ethylacetate and acetone) or vehicle. The cells were pulsed with 1 µCi ^{3}H-thymidine for the last 4 hours. Results (mean of triplicates ± SEM) show the number of ^{3}H-thymidine cpm counted per well.

Student's t test : difference with cells treated with vehicle , * $p < 0.05$; ** $p < 0.01$.

2A: Hexane

2B: Hexane/Ethylacetate (10/1)

2C: Hexane/Ethylacetate (5/1)

2D: Ethylacetate

2E: Acetone.

Results show that these fractions of extract stimulate cell proliferation. In order to compare the effects of our extract to commercial phyto-estrogens, we tested the effects of daidzein on the proliferation of the ROS osteoblastic cells. Results show that daidzein does not have any effect on the proliferation of the ROS osteoblastic cells (Data not shown). This result confirms that of Kanno *et al.* (2002) who showed that although daidzein positively affects bone metabolism, it does not have any effect on the proliferation of MC3T3-E1 cells. This is also supported by De wilde *et al.* (2001). But other authors showed that daidzein stimulates cell proliferation (Cho *et al.*, 2002). However, the different outcomes observed in these studies may be due to the different cell types used. On the other hand, estrogenic substances are known to have some dose-dependent effects. Thus, the effects of estrogenic substances can be agonistic to those of estrogens at low dose and antagonistic at high dose. Besides, the stimulative effects of the estrogens depend, among others, on cell type and dose used (Kelly *et al.*, 1995; Xu *et al.*, 1995; Wiseman, 1999).

We studied the effects of our extracts in combination with partial inhibitor of estrogens such as Tamoxifen which blocks estrogen receptors. The results show no effect of Tamoxifen on the stimulative effects induced by the hydro-ethanolic extract of *Phaseolus vulgaris*. This suggests that the substances in these extracts do

not use estrogen receptors in their mechanisms of action, although they are estrogenic compounds. Indeed, phyto-estrogens can act through other non genomic mechanisms not requiring estrogens receptors (Kurzer and Xu, 1997). Phyto-estrogens can interact with enzymes and receptors (Adlercreutz, 1998). On the other hand, they may be in the same extracts substances that are not phyto-estrogens but that can act on cell proliferation.

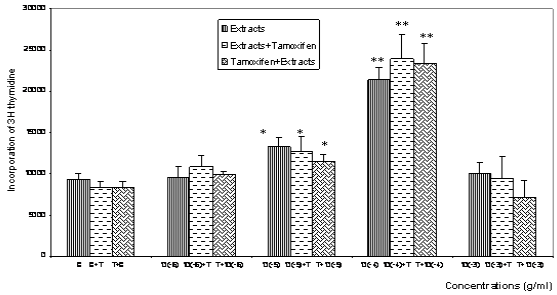

Fig. 3. Modulation by tamoxifen of the effect of hydro-ethanolic extracts on ROS 17/2.8 proliferation.

ROS 17/2.8 cells were incubated for 48 hours in MEM supplemented with 1 mg/ml Albumax supplemented with different concentrations of hydro-ethanolic plant extracts or vehicle. 10^{-6} g/ml of Tamoxifen was added in the culture medium 30 minutes either before or after the addition of the extracts. The cells were pulsed with 1 µCi ^{3}H-thymidine for the last 4 hours. Results (mean of triplicates ± SEM) show the number of ^{3}H-thymidine cpm counted per well.

Student's t test : difference with cells treated with vehicle , * $p < 0.05$; ** $p < 0.01$.

Tamoxifen does not suppress the effects of the acetonic extracts although it reduces them. This reduction is stronger for low dose of extract. This also suggests that the substances always use partially estrogens receptor. The different effect observed between hydro-ethanolic and acetonic extracts may be due to the presence of more estrogens receptors-using substances in the latter so that their effects are likely to be blocked by Tamoxifen. The hydro-ethanolic extract probably uses other mechanisms to act on the proliferation of osteoblastic cells.

We also analyzed the effects of *Phaseolus vulgaris* extract on the differentiation of ROS osteoblastic cells. The results show that the hydro-ethanolic extract activates cell differentiation. Our results confirm those obtained by many authors with different.

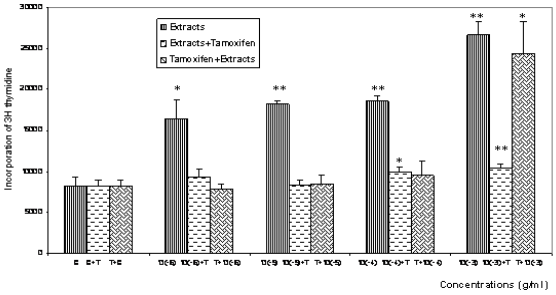

Fig. 4. Modulation by tamoxifen of the effect of acetonic extracts on ROS 17/2.8 proliferation.

ROS 17/2.8 cells were incubated for 48 hours in MEM supplemented with 1 mg/ml Albumax supplemented with different concentrations of acetonic plant extracts or vehicle. 10^{-6} g/ml of Tamoxifen was added in the culture medium 30 minutes before or after the addition of the extracts. The cells were pulsed with 1 µCi ^{3}H-thymidine for the last 4 hours. Results (mean of triplicates ± SEM) figurate the number of ^{3}H-thymidine cpm counted per well.

Student's t test : difference with cells treated with vehicle , * $p < 0.05$; ** $p < 0.01$.

plants. Indeed, Jeong *et al.* (2005) showed that extracts of *Drynariae rhizoma* stimulate the proliferation and the differentiation of osteoblastic MC3T3-E1 cells. Oh *et al.* (2003) obtained the same results with extracts of *Rehmannia glutinosa* Libosch.

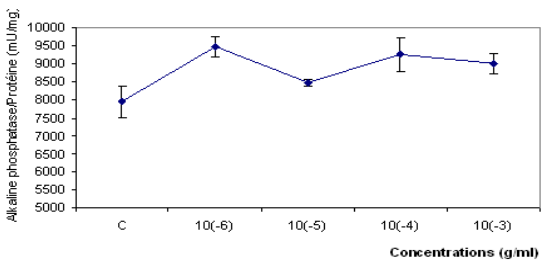

Fig. 5. Effect of hydro-ethanolic extracts on alkaline phosphatase levels of ROS 17/2.8 cells.

Osteoblast-like cells were incubated for 48 hours in MEM supplemented with 1 mg/ml Albumax with different concentrations of plant extracts or vehicle.
At the end of the incubation, levels of alkaline phosphatase were measured. Results (mean of triplicates ± SEM) are expressed in mU / mg protein.
Student's t test : difference with cells treated with vehicle , * $p < 0.05$; ** $p < 0.01$

Acetonic extracts of *Phaseolus vulgaris* do not have any effect on the differentiation of ROS osteoblastic cells. The daidzein stimulated the differentiation (Data not shown) which is similar to results obtained by Sugimoto and Yamagushi (2000) with daidzein on MC3T3-E1cells. Other authors obtained similar results with daidzein (Cho *et al.* 2002; Dang *et al.*, 2002; Yamagushi and Weitzmann, 2009). We investigated the effects of our extracts on the differentiation of osteoblastic cells in presence of Tamoxifen. The results show that the presence of Tamoxifen totally inhibits the effects induced by plants extracts. These results are similar to those published by many authors on the topic. Indeed, Choi *et al.* (2001) showed that the stimulation induced by an ethanolic extract of *Glycine max* seeds was blocked completely by Tamoxifen. A similar result is obtained whith extracts of *Drynaria rhizoma* (Jeong *et al.*, 2005). Our results suggest that the stimulation of the differentiation by extracts of *Phaseolus vulgaris* is made through estrogens receptors that carry osteoblastic cells.

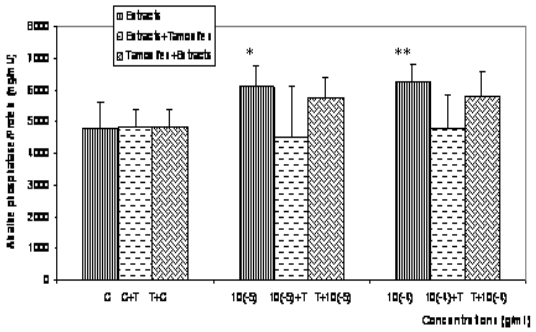

Fig. 6. Modulation by tamoxifen of the effect of hydro-ethanolic extracts on alkaline phosphatase levels in ROS 17/2.8.

ROS 17/2.8 cells were incubated for 48 hours in MEM supplemented with 1 mg/ml Albumax supplemented with different concentrations of hydro-ethanolic plant extracts or vehicle. 10^{-6} g/ml of Tamoxifen was added in the culture medium 30 minutes before or after the addition of the extracts.

At the end of the incubation, levels of alkaline phosphatase were measured. Results (mean of triplicates ± SEM) are expressed in mU / mg protein.
Student's t test: difference with cells treated with vehicle, * $p < 0.05$; ** $p < 0.01$

We noticed that Tamoxifen inhibits the differentiation induced by the extracts on whereas the inhibitory effect of the Tamoxifen on the proliferation is only partial or even non-existent. This difference may result from the different mechanisms used to stimulate these two physiological processes. Indeed, the activation of the differentiation may essentially be due to the action of phyto-estrogens using estrogens receptors exclusively. The proliferation may also involve other substances and/or phyto-estrogens using other molecular mechanisms that do not involve estrogen receptors. These mechanisms become predominant on the possible estrogen receptor utilization.

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References

Adlercreutz H. 1998. Evolution, nutrition, intestinal microflora and prevention of cancer: a hypothesis. Proceedings of the Society of Experimental Biology and Medicine **217,** 241-247.

Arjmandi BH, Khalil DA., Smith BJ, Lucas EA, Juma S, Payton ME, WILD RA. 2003. Soy protein has a greater effect on bone in postmenopausal women not on hormone replacement therapy, as evidenced by reducing bone resorption and urinary Calcium excretion. The Journal of

Clinical Endocrinology and Metabolism **88(3)**, 1048–1054.
http://dx.doi.org/10.1210/jc.2002-020849

Benié T, Thieulant M-L. 2003. Interaction of some traditional plant extracts with uterine estrogen or progestin receptors. Phytotherapy research **17**, 756-760.
http://dx.doi.org/10.1002/ptr.1208

Boue SM, Wiese TE, Nehls S, Burrow ME, Elliott S, Carter-Wientjes CH, Shih BY, Maclachlan JA, Cleveland TE. 2003. Evaluation of the estrogenic effects of legume extracts containing phytoestrogens. Journal of Agricultural Food Chemistry **51**. 2193-2199.
http://dx.doi.org/10.1021/jf021114s

Chen X, Anderson JJB. 2002. Effects of isoflavones on osteoblast proliferation and differentiation. Journal of Nutrition **132**, 616S

Choi EM, Suh KS, Kim YS, Choue RW, Koo SJ. 2001. Soybean ethanol extract increases the function of osteoblastic MC3T3-E1 cells. Phytochemistry **56**, 733-739.
http://dx.doi.org/10.1016/s0031-9422(00)00484-2

Choi EM. 2005. The licorice root derived isoflavan glabridin increases the function of osteoblastic MC3T3-E1 cells. Biochemical Pharmacology **45**, 698-704.

Cho Y, Chung S, Cho K. 2002. Black bean (Rhynchosia molubilis, Yak-kong) exerts a prominent estrogenic effect on the proliferation of human MG63 osteoblastic cells. Journal of Nutrition **132**, 614-s

Dang ZC, Papapoulos S, Löwik C. 2002. Phyto-estrogens enhance osteogenegis and concurrently inhibit adipogenesis. Journal of Nutrition **132**, 617-s.

Draper R, Edel M, Dick I, Randall A, Martin G, Prince R. 1997. Phytoestrogens Reduce Bone Loss and Bone Resorption in Oophorectomized Rats1. American Society for Nutritional Sciences, 1795-1799.

De wilde A, Colin C, Lacroix H. 2001. In vitro and ex vivo effects of an isoflavone-rich diet on bone formation in growing pigs. Annal of Nutrition Metabplism **45**, 227-S.

Duque A, Rakic P. 2011. Different effects of bromodeoxyuridine and 3H-thymidine incorporation into DNA on cell proliferation, position and fate. The journal of Neurosciences **31(42),** 15205-15217.
http://dx.doi.org/10.1523/jneurosci.3092-11.2011

Egrise D, Martin D, Neve P, Verhas M, Schoutens A. 1990. Effects and interactions of 17β-estradiol, T3 and 1,25(OH)2D3 on cultured osteoblasts from mature rats. Bone and mineral **11**, 273-283.

Egrise D, Martin D, Neve P, Vienne A, Verhas M, Schoutens A. 1992. Bone blood flow and In vitro proliferation of bone marrow and trabecular bone osteoblast-like cells in ovariectomized rats. Calcified Tissue International **50**, 336-341.

Janeczko A, Skoczowski A. 2005. Mammalian sex hormones in plants. Folia Histochemica et Cytobiologica **43(2)**, 71-79.

Jeong J-C, Lee JW, Yoon CH, Lee YC, Chung KH, Kim MG, Kim CH. 2005. Stimulative effects of Drynariae rhyzoma extracts on the prolifeation and differentiation of osteoblastic MC3T3-E1 cells. Journal of Ethnopharmacology **96(3)**, 489-495.
http://dx.doi.org/10.1016/j.jep.2004.09.038

Kanno S, Hirano S, Kayama F. 2004. Effects of phytoestrogens and environmental estrogens on osteoblastic differentiation MC3T3-E1 cells. Toxicology **196**, 137-145.
http://dx.doi.org/10.1016/j.tox.2003.12.002

Kelly GE, Joannou GE, Reeder AY, Nelson C, Waring MA. 1995. The variable metabolic response

to dietary isoflavones in humans. Proceedings of the Society of Experimental Biology and Medicine **208**, 40-43.

Kong J-M, Chia L-S, Goh N-K, Chia T-F, Brouillard R. 2003. Analysis and biological activities of anthocyanins. Phytochemistry **64**, 923-933. http://dx.doi.org/10.1016/s00319422(03)00438-2

Kopcewicz J. 1971. Estrogen in developing bean (*Phaseolus vulgaris*) plants Phytochemestry **10**, 1423-1427

Kurzer MS, Xu X. 1997. Dietary phytoestrogens, Annual Revue of Nutrition **17**, 353-381. http://dx.doi.org/10.1146/annurev.nutr.17.1.353

Lee YB, Lee HJ, Kim KS, Lee JY, Nam SY, Cheon SH, Sohns HS. 2004. Evaluation of the preventive effect of isoflavone extract on bone loss in ovariectomized rats. Biosciences Biotechnology and Biochemistry **68(5)**, 1040-1045. http://dx.doi.org/10.1271/bbb.68.1040

Mazur W. 1998. Phytoestrogen content in foods, Baillieres Clinical Endocrinology Metabolism **12**, 729-742.

Mizutani K, Ikeda K, Kawai Y, Yamori Y. 1998. Resveratrol stimulates the proliferation and differentiation of osteoblastics MC3T3-E1 cells. Biochemical and Biophysical Resources Communication **253**, 859-863. http://dx.doi.org/10.1006/bbrc.1998.9870

Morabito N, Crisafulli A, Vergara C, Gaudio A, Lasco A, Frisina N, D'anna R, Corrado F, Pizzoleo MA, Cincotta, Altavilla D, Lentile R, Squadrito F. 2002. Effects of genistein and hormone-replacement therapy on bone loss in early

postmenopausal women: a randomized double-blind placebo-controlled study. Journal Bone Mineral Resource **17**, 1904–1912. http://dx.doi.org/10.1359/jbmr.2002.17.10.1904

Oh K-O, Kim S-W, Kim J-Y, Ko S-Y, Kim H-M, Baek J-H, Ryoo H-M, Kim J-K. 2003. Effect of *Rehmannia glutinosa* Libosch extracts on bone metabolism. Clinica chimica Acta 334, 185-195. http://dx.doi.org/10.1016/s0009-8981(03)00238-9

Ogata H, Hayashi M, Tsuda H, Suzuki N, Maeno M, Sugawara A, Ogiso B. 2012. Effects of calcium phosphate cement on ineralized nodule formation compared with endodontic cements. Dental materials Journal **31(1),** 92-97. http://dx.doi.org/10.4012/dmj.2011-151

Ososki AL, Kennely EJ. 2003. Phytoestrogens: a review of the present state of research. Phytotherapy Research **17**, 845-869. http://dx.doi.org/10.1002/ptr.1364

Om AS, Shim JY. 2007. Effects of daidzein, a soy isoflavone, on bone metabolism in cd-treated ovariectomized rats. Acta Biochimica Polonica **54(3)**, 641-647.

Pari L, Venkateswaran S. 2003. Effect of an aqueous extract of *Phaseolus vulgaris* on the properties of tail tendon collagen of rats with streptozotocin-induced diabetes. Brazilian Journal of Medical and Biological Research **36**, 861-870. http://dx.doi.org/10.1590/s0100-879x2003000700006

Rassi CM, Lieberherr M, Chaumaz G, Pointillart A, Cournot G. 2002. Down-regulation of osteoclast differentiation by daidzein via caspase 3. Journal of Bone Mineral Resources **17(4)**, 630-638. http://dx.doi.org/10.1359/jbmr.2002.17.4.630

Reynoso-Camacho R, Ramos-Gomez M, Loarca-Pina G. 2006. Bioactive components in common beans (*Phaseolus vulgaris* L.). Advances in Agricultural and food Biotechnology, 217-236.

Shirke SS, Jadhav SR, Jaqtap AG. 2009. Oteoprotective effects of *Phaseolus vulgaris* L. in ovariectomized induced osteopenia in rats. Menopause **16(3),** 589-596.
http://dx.doi.org/10.1097/gme.0b013e31818e64c4

Sugimoto E, Yamaguchi M. 2000. Anabolic effect of genistein in osteoblastic MC3T3-E1 cells. International Journal of Molecular Medicine **5(5)**, 515-520.

Wiseman H. 1999. The bioavailability of non-nutrient plant factors: dietary flavonoids and phyto-estrogens, Proceedings of the Society of Experimental Biology and Medicine **58**, 139-146.
http://dx.doi.org/10-1079/pns19990019

Xu X, Harris KS, Wang H-J, Murphy PA, Hendrich S. 1995. Biovailability of soybean isoflavones depends upon gut microflora in women. Journal of Nutrition **125**, 2307-2315.

Yamagushi M, Weitzmann MN. 2009. The estrogen 17β-estradiol and phytoestrogen genistein mediate differential effects on osteoblastic NF-κB activity. International Journal of Molecular Medicine **23(2),** 297-301.
http://dx.doi.org/10.3892/ijmm_00000131

Ye S-F, Saga I, Ichimura K, Nagai T, Shinoda M, Matsuzaki S. 2003. Coumestrol as well as isoflavones in soybean extract prevent bone resorption in ovariectomized rats. Endocrine regulations **37(3)**, 145-152.