TO STUDY THE REGULATION OF BONE MARKERS IN OSTEOPOROSIS

THESIS

SUBMITTED FOR THE AWARD OF THE DEGREE OF

Doctor of Philosophy

IN

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BY

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Dated:..................
Approved:.............

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The word “osteoporosis” means “porous bone”. Bone is living, growing tissue that changes throughout the lifespan. Osteoporosis is a progressive and chronic disease of the skeleton characterized by bone fragility, which is because of reduction in bone mass as well as possible alterations in bone architecture. This in turn, consequently, leads to a propensity for fractures with minimum trauma.

The formation of bone is related to osteoblastic proliferation, osteocalcin, and alkaline phosphatase (ALP) activity, as well as collagen synthesis, while bone resorption is associated with osteoclast formation and differentiation as well as tartrate resistant acid phosphatase (TRAP) activity. It’s globally well documented that osteoporosis and associated fractures constitute a major public health issue together representing an important cause of mortality and morbidity with annual incidence of fracture rates exceeding the combined incidence of breast cancer, stroke and heart attack in postmenopausal women (PMW). It may be worth in pointing out here that the emergence of osteoporosis as a global bone health concern is reflected in the occurrence of 1.6 million hip fractures annually at the global level, and the number projected may go up around four times high by year 2050-2055. Osteoporosis affects both men and women; however the later are more susceptible targets of this crippling disorder of bone.

It’s well established that oxidative stress is the outcome due of imbalance between the generation of reactive oxygen species (ROS) and the activity of antioxidant defense system. Intense oxidative stress has been implicated in many chronic and degenerative diseases, including cancer, ageing, osteoporosis, and neurodegenerative diseases such as Parkinson’s disease, Alzheimer’s disease, and amyotrophic lateral sclerosis. Several medications such as calcium products, estrogen, bisphosphonates, ipriflavone and anabolic steroids have been reported to be effective for curing osteoporosis. However, these medications may have serious side effects, may not improve bone quality, or may not reduce susceptibility to fracture.
Abstract

It may be noteworthy in pointing out here that reactive oxygen species (ROS) can/may play an important role in bone loss in patients with osteoporosis by generating a more oxidized bone microenvironment. Worldwide, osteoporosis is known since the origin of human civilization. Unfortunately, till date, a clear cut complete understanding about its management still remains poorly understood. Augmented generation of ROS in vivo due to a wide reason concerned with in-vivo conditions, leads to the activation and up-regulation of bone markers like pro-inflammatory cytokine TNF-α and its super family member OPG as well as OPN and calcitonin (CT) at both the gene i.e. mRNA and protein levels. This in turn results in accelerated osteoclast differentiation, thereby resulting to loss of bone mass, including osteoporosis, arthritis, orthopaedic implant loosening, etc. Thus, if ROS production in vivo is regulated by natural antioxidants, then the above-mentioned bone markers associated with chronic bone conditions may/can be easily managed/regulated.

Wide spectrum of medical treatments such as calcitonin, anabolic steroids, calcium products, bisphosphonates, estrogen, and ipriflavone has been shown to be effective for the remedy of osteoporosis. However, despite of the beneficial effects of the above remedial agents, they have also been reported to have serious side effects, which probably may not improve bone quality, or may not reduce susceptibility to fracture. As a matter of fact, since last one decade, alternative medicines have been subjected to in-depth probe by doctors/clinicians as these alternative medicines from natural sources have shown fewer side effects and are more suitable for long-term use as compared to modern medicines synthesized chemically. In view of it, researches at global levels have shown that some natural flavonoids/antioxidants with potent antioxidant activity including resveratrol, scopoletin, and baicalein have found to exert anti-osteoporotic activities through suppressing osteoclast formation and TRAP. Accordingly, natural antioxidants have become a topic of increasing interest among basic and clinical scientists as they could provide a safe, economical and valuable tool in combating such diseases.

Recent investigations have revealed that T lymphocytes and their product have also been recognized as key regulators of bone cell formation, lifespan and activity. Reports indicate a number of pro-inflammatory cytokines to be activated in osteoporosis. However, TNF-α, the autocrine cytokine, is perhaps the most dominant
Abstract

cytokine, which promotes osteoclastogenesis by augmenting the production of RANKL, the non-redundant cytokine responsible for osteoclast development. And that, this type of capability of autocrine cytokine TNF-α, is solely due to the synergistic interactions at the level of NF-κB and activator protein-1 (AP-1) signalling. Apart from the above, TNF-α stimulates osteoclast (OC) activity and inhibits osteoblastogenesis, thereby, further driving an imbalance between bone formation and bone resorption. It’s well established that globally, there is ever increasing number of elderly people, improved survival and an increase in the age-specific fracture rates; thus, there ought to be an immediate need to develop new cost-effective therapeutic strategies against osteoporosis. Therefore, in the present study, natural compounds like punicalagin, resveratrol, quercetin as well as ascorbic acid were employed as valuable natural antioxidants/natural tools in order to investigate the above.

Punicalagin from pomegranate, resveratrol from grapes and quercetin from onions are the major biologically active polyphenolic compounds, whereas ascorbic acid (vitamin C) rich in citrus fruits have shown potent antioxidant properties. They have been reported to possess potent anticancer properties besides showing broad range anticancer and antifungal activities. Besides, they possess radical scavenging properties in activated granulocytes and may also inhibit iNOS expression in activated macrophages.

Because of the established fact that reactive oxygen species (ROS) and reactive nitrogen species (RNI) playing an important role in both estrogen related and unrelated osteoporosis, thus, greater emphasis now being laid on development of compounds from natural sources having antioxidant and anti-inflammatory properties in combating osteoporosis.

The first part of our study shows an attempt to probe and carry out characterization of monocytes of osteoporosis patients in order to assess the activity of predominant antioxidant enzyme, GPx as well as to measure the intramonocyte (GSH) levels. Glutathione (GSH) directly reacts with ROS and glutathione peroxidase (GPx) catalyses the removal of hydrogen peroxide. Our ELISA results exhibited nearly less than half the activity of enzyme in monocytes of osteoporosis patients as
Abstract

compared to healthy individuals. Suppressed/decrease in GPx activity indicates
impairment of hydrogen peroxide-neutralizing mechanism. Likewise, suppression in
intramonicocyte GSH levels was also observed in these patients, which in turn, were
indicative of weak antioxidant power. Furthermore, elevated free radicals in
osteoporosis patients were also substantiated by MDA determination.

Our study showed that the levels of by-product of lipid peroxidation namely
malondialdehyde (MDA), to be appreciably high in monocytes cultures of
osteoporosis patients, thereby, further pointing to the augmented/increased oxidant
stress in such patients. An increase in hydrogen peroxide levels due to depressed GSH
levels and GPx activity in osteoporosis patients might have induced the peroxidation
of polyunsaturated fatty acids, thereby leading to the formation of high MDA levels.
Moreover, due to high reactivity of MDA towards amino groups may also lead to
deactivation of enzymes. Punicalagin, resveratrol, quercetin and ascorbic acid dose-
dependently down-regulated the excess levels of MDA in monocytes cultures of
osteoporosis patients.

Scores of investigations/studies carried out globally show established evidences
that estrogen prevents bone loss by blocking the production of pro-inflammatory
cytokines by bone marrow stromal and bone cells. The most prominent cytokines that
are regulated by estrogen are IL-1β, IL-6 and TNF-α. In the present study also, probe
by ELISA exhibited an appreciable secretion of TNF-α and IL-1β in monocytes of
osteoporosis patients. In fact, IL-1β is a potent stimulator of bone resorption together
with TNF-α, and both are well-recognized inhibitors of bone formation. IL-1β and
TNF-α are also powerful inducers of other cytokines such as IL-6, M-CSF and GM-
CSF, which potentiate the effect of IL-1β on osteoclastogenesis.

Thereafter, monocytes from osteoporosis patients were also characterized with
respect to TNF-α and IL-1β, wherein monocytes from these patients evaluated by
ELISA, which revealed appreciably high basal levels of both TNF-α and IL-1β
expressions in comparison to healthy controls.

Following the above results exhibiting impaired antioxidant systems in
osteoporosis patients as revealed by decreased GPx activity and reduced GSH levels,
Abstract

An attempt was made to have further insight by measuring the levels of a by-product of lipid peroxidation i.e., malondialdehyde (MDA) in the 24 hours monocytes cultures. This helped to detect further signs of increased oxidative stress.

In comparison to healthy group (10.11 ng/ml), the MDA values were found to be almost 2.9 times higher in osteoporosis patients (29.36 ng/ml). Co-culturing separately of healthy monocytes as well as patient’s monocytes with 2 nM H$_2$O$_2$ yielded augmented MDA expressions, where the MDA levels were found to be 17.26 ng/ml and 37.18 ng/ml respectively. Around 1.7 times and 3.67-times augmented MDA levels were found in H$_2$O$_2$ treated healthy and patient’s monocytes respectively, thereby indicating H$_2$O$_2$ mediated/induced augmented oxidative stress in healthy cells, and that, it further augmented in patient’s cells. MDA expressions in untreated healthy monocytes, 10 M NAC-treated healthy monocytes, untreated patient’s monocytes and 10 nM NAC-treated patient’s monocytes were recorded to be 10.98 ng/ml, 11.21 ng/ml, 28.19 ng/ml and 19.03 ng/ml respectively. The down-regulation/suppression of MDA levels in NAC-treated patient’s cells by around 67.5 percent is indicative for the appreciable containment/arrest of oxidative stress in patient’s cells.

Prior to any experiments, an attempt was made to study the toxic effect of natural antioxidants/polyphenols employed in the study on human monocytes by means of MTT cell viability assay. The cell viability assays revealed that at 24 hours culture, no adverse/toxic effect on monocytes was observed with doses of 0-100 µg/ml of quercetin, resveratrol, punicalagin and vitamin C respectively. However, cell viability was affected in 48 hours cultures at doses at or above 50 µg/ml of quercetin and resveratrol, and nearly the same observation was made in 72 hours monocytes cultures treated with punicalagin. However, ascorbic acid (vitamin C) had no adverse effect on cell viability on all the four days (i.e. 24 hours, 48 hours, 72 hours and 120 hours) of cell cultures.

An attempt was made to probe the effect of varying doses of quercetin, punicalagin, resveratrol and ascorbic acid (0, 2, 5, 10, 15 and 20 µg/ml) on the status of intramunocyte GSH in monocytes cultures (24 hours). Significantly suppressed/down-regulated levels (109.44 pg/ml) of GSH were recorded in cultures devoid of any supplement in patients cells when compared to normal healthy subjects.
(297.95 pg/ml). Thereafter, the GSH expression increased dose-dependently with all the four supplements i.e. quercetin, punicalagin, resveratrol and ascorbic acid. With resveratrol, the amelioration in GSH levels were recorded as 138.22, 144.14, 223.42, 240.17 and 270.52 pg/ml with 2, 5, 10, 15 and 20 µg/ml resveratrol respectively. Next, with quercetin, the amelioration in GSH levels were recorded as 122.13, 131.65, 201.25, 223.76 and 256.29 pg/ml with 2, 5, 10, 15 and 20 µg/ml quercetin respectively. Thereafter, the effect of varying doses of ascorbic acid (vitamin C) was evaluated. Amelioration in GSH levels were recorded as 115.24, 127.34, 187.26, 214.87 and 233.96 pg/ml with 2, 5, 10, 15 and 20 µg/ml ascorbic acid respectively. Interestingly, a slightly higher amelioration effect in GSH expression was observed with punicalagin. None of these above mentioned supplements showed any effect on healthy normal monocytes culture. Therefore, natural antioxidants may be effectively used to improve the degenerating antioxidant state in the pathogenesis of osteoporosis.

Adherent monocytes from PBMCs were cultured for 24 hours. Thereafter, the collected supernatants were analyzed for MDA levels. Analysis of the data obtained revealed that in osteoporosis patients the MDA levels stood to a near 5.68 times (39.71 ng/ml) the level found in healthy group (6.98 ng/ml). Our data exhibiting extremely augmented MDA levels are indicative for the fact that osteoporotic patients are exposed to high oxidative stress.

Monocytes from both healthy subjects and osteoporosis patients were co-cultured for 24 hours with varying doses of punicalagin, resveratrol, ascorbic acid and quercetin (0-25 µg/ml), and subsequently, the supernatants were subjected to evaluation for MDA levels. None of the doses of punicalagin, resveratrol, ascorbic acid and quercetin had any effect on the MDA levels in the supernatants of monocytes culture of healthy subjects, where it was recorded in the range of range of 6.03 ng/ml to 5.77 ng/ml. On the contrary, in cell cultures of osteoporosis patients, the MDA levels showed a dose-dependent decrease with all the above four mentioned supplements.

The presence of augmented levels of pro-inflammatory cytokines like TNF-α and IL-1β in osteoporosis has been widely reported by scores of workers. Therefore,
in this study also, an attempt was made to evaluate the levels of TNF-α and IL-1β in monocytes culture supernatants of osteoporosis patients, and in turn, results were compared with those obtained from healthy subjects. Appreciably augmented basal levels of TNF-α and IL-1β were recorded in monocytes culture supernatants of osteoporosis patients (172.33 pg/ml and 140.98 pg/ml respectively) compared to healthy control cells (4.98 and 7.04 pg/ml respectively).

The effect of varying doses (0-100 µg/ml) of punicalagin, resveratrol, ascorbic acid and quercetin on the expressions of TNF-α and IL-1β in 24 hours monocytes culture supernatants by ELISA showed interesting results. Secretion of TNF-α and IL-1β were found to decrease/down-regulated dose-dependently with all the above mentioned supplements in osteoporosis patients.

In accordance to earlier reports from our laboratory, again this study showed that after the 3 days culture duration in osteoclastogenic medium, the multinucleated osteoclast precursors were observed to appear. The osteoclastogenic medium was α-MEM culture medium that was supplemented with 10% FCS, 100 U/ml penicillin, 100 µg/ml streptomycin, 50 ng/ml M-CSF and 25 ng/ml RANKL. Thereafter, the number of such multinucleated cells was found to increase after 5 days of culture, as demonstrated by tartrate resistant acid phosphatase (TRAP) staining. Interestingly, there was no or negligible appearance of osteoclast precursors after 24 hours (1 day) of culture.

It was observed that co-culturing of PBMCs with punicalagin (20 µg/ml) or resveratrol (20 µg/ml) or quercetin (20 µg/ml) or ascorbic acid (20 µg/ml), in osteoclastogenic medium for 3 and 5 days resulted in an appreciable amount of reduction in appearance of multinucleated osteoclast precursors. The above doses of punicalagin, resveratrol, quercetin and ascorbic acid were selected after performing dose response experiment, where TRAP assay revealed a linear suppression in the formation of multinucleated cells was observed. Hence, our data is indicative towards the potential of the above supplements having antioxidant property to exert effective regulatory effect in osteoclast generation and differentiation.

Next, an attempt was also made to study the expression at protein levels of sRANKL in culture supernatants of healthy controls and osteoporosis patients by
ELISA, where our data revealed an expression of around 6.8-fold augmented levels of sRANKL (28.88 pg/ml) in comparison to healthy controls (4.21 pg/ml).

Adherent mononuclear cells obtained from osteoporosis patients, were co-cultured with varying concentrations of punicalagin, resveratrol, quercetin and ascorbic acid (0-25 µg/ml) and levels of sRANKL in culture supernatants.

With varying doses of punicalagin, the sRANKL secretion in patient’s cell culture was recorded to be dose-dependently suppressed/down-regulated from 28.88 pg/ml to 21.36, 12.67, 8.39 5.12 and 4.28 pg/ml with 0, 5, 10, 15, 20 and 25 µg/ml of punicalagin, respectively. On the other hand, the cells of healthy control subjects that were co-cultured with varying doses of punicalagin, showed sRANKL levels to the order of 2.49, 2.76, 2.18, 3.16, 2.99 and 2.27 pg/ml with 0, 5, 10, 15, 20 and 25 µg/ml punicalagin respectively. Thus, none of the selected doses of punicalagin showed any effect in healthy control cells.

With varying doses of resveratrol, the sRANKL expression was found to be dose-dependently suppressed/inhibited from 28.88 pg/ml to 22.76, 14.23, 10.56, 6.43 and 4.97 pg/ml with 0, 5, 10, 15, 20 and 25 µg/ml of resveratrol, respectively. However, when compared to the above data obtained from patient’s cells, it was observed that the cells of healthy control subjects which were co-cultured with varying doses of the above said supplement, displayed sRANKL levels to the order of 3.88, 1.31, 2.83, 3.09, 2.88 and 2.16 pg/ml with 0, 5, 10, 15, 20 and 25 µg/ml resveratrol respectively. Thus, none of the selected doses of resveratrol showed any effect in healthy control cells.

With varying doses of ascorbic acid (vitamin C), it was observed that sRANKL secretion was dose-dependently down-regulated from 28.88 pg/ml to 22.89, 16.34, 11.53, 9.78 and 6.77 pg/ml with 0, 5, 10, 15, 20 and 25 µg/ml of ascorbic acid, respectively. Mononuclear cells of healthy control subjects that were co-cultured with varying doses of ascorbic acid, exhibited sRANKL levels to the order of 2.56, 2.98, 3.12, 3.89, 2.19 and 2.67 pg/ml with 0, 5, 10, 15, 20 and 25 µg/ml quercetin respectively. Thus, none of the selected doses of ascorbic acid showed any effect in healthy control cells.
With varying doses of quercetin, it was found here that in cultures of patient’s cells, sRANKL secretion was dose-dependently suppressed/down-regulated from 28.88 pg/ml to 23.19, 15.89, 11.04, 8.99 and 6.89 pg/ml with 0, 5, 10, 15, 20 and 25 µg/ml of quercetin, respectively. However, contrary to the above, the cells of healthy control subjects that were co-cultured with varying doses of the above said supplement, displayed sRANKL levels to the order of 3.88, 1.31, 2.83, 3.09, 2.88 and 2.16 pg/ml with 0, 5, 10, 15, 20 and 25 µg/ml quercetin respectively. Thus, none of the selected doses of quercetin showed any effect in healthy control cells.

Computational analysis for supplement-induced percent inhibition in the expression of sRANKL revealed that a linear dose-dependent inhibition of sRANKL expression occurred with all the four selected natural antioxidants/polyphenols employed in our study.

Modulation of sRANKL in the presence or absence of TNF-α (2 ng/ml) along with either punicalagin (20 µg/ml) or resveratrol (20 µg/ml), or ascorbic acid (20 µg/ml) or quercetin (20 µg/ml) revealed that there was negligible effect of TNF-α alone or with a combination of both TNF-α and punicalgin or resveratrol or ascorbic acid or quercetin on healthy control cultures in comparison to untreated cultures. In comparison to control cells, in patient’s cultures, the sRANKL levels was recorded to be of the order of 30.67 pg/ml, and that, this was up-regulated/augmented by TNF-α (2 ng/ml) to the order of 47.81 pg/ml. Encouraging results were obtained with a combination of TNF-α (2 ng/ml) and punicalagin (20 µg/ml) or resveratrol (20 µg/ml) or ascorbic acid (20 µg/ml) or quercetin (20 µg/ml), where appreciable down-regulation in sRANKL levels were recorded, which were of the order of 10.32 pg/ml, 12.87 pg/ml, 13.45 pg/ml and 13.50 pg/ml respectively. Thus, the computational analysis revealed that secretion of sRANKL was inhibited by around 60%, 50%, 45% and 40% by punicalagin, resveratrol, ascorbic acid and quercetin respectively.

Next, the expression of osteopontin (OPN) in monocytes culture supernatants of healthy controls and osteoporosis patients was determined by ELISA. In 3 days (72 hours) culture supernatants of monocytes obtained from healthy control, no or negligible levels (2.56 ng/ml) of OPN were observed, while a 6.01-fold augmented
Abstract

OPN was detected in 72 hours culture supernatants of monocytes from osteoporosis patients (15.41 ng/ml).

Modulation study of OPN was carried out where monocytes from osteoporosis patients were cultured for 3 and 5 days in osteoclastogenic medium with and without 1 ng/ml of calcitonin (CT). Thus, cultures devoid of any CT revealed OPN levels to the order of 22.14 ng/ml, while cultures receiving 1 ng/ml CT exhibited an appreciably augmented expression (~1.7-fold), which was of the order of 38.12 ng/ml in 5 days culture. The presents data substantiates that calcitonin was a positive modulator of osteopontin.

Modulation with punicalagin + calcitonin (CT) (1 ng/ml) exhibited an appreciably suppressed levels of OPN in monocytes cultures receiving 20 µg/ml of punicalagin (5.67 ng/ml). Next, cultures receiving a combination of punicalagin and CT as mentioned above showed suppressed OPN levels which were of the order of 10.02 ng/ml. Our data clearly exhibits the positive modulatory effect of CT on OPN was appreciably neutralized by punicalagin. This indicates that punicalagin was exerting a remarkable negative modulatory effect on OPN.

Modulation with resveratrol + calcitonin (CT) exhibited appreciably suppressed levels of OPN in monocytes culture receiving 20 µg/ml of resveratrol (6.93 ng/ml). Next, cultures receiving a combination of resveratrol and CT, showed suppressed OPN levels which were of the order of 12.54 ng/ml. The results clearly exhibited the positive modulatory effect of CT on OPN was appreciably neutralized by resveratrol. This indicates that resveratrol was exerting an appreciable negative modulatory effect on OPN.

Modulation with ascorbic acid + calcitonin (CT) showed appreciably suppressed levels of OPN in monocytes culture receiving 20 µg/ml of ascorbic acid (7.22 ng/ml). Next, cultures receiving a combination of ascorbic acid and CT showed suppressed OPN levels which were of the order of 13.12 ng/ml. Again, the results clearly exhibited the positive modulatory effect of CT on OPN was appreciably neutralized by ascorbic acid. Thus, ascorbic acid was exerting an appreciable negative modulatory effect on OPN.
Modulation with quercetin + calcitonin (CT) revealed an appreciably suppressed level of OPN in monocytes culture receiving 20 µg/ml of quercetin (7.87 ng/ml). Next, cultures receiving a combination of quercetin and CT as mentioned above, showed suppressed OPN levels which were of the order of 13.92 ng/ml, and therefore the positive modulatory effect of calcitonin on osteopontin was appreciably neutralized by quercetin, and that, quercetin was hence exerting an appreciable negative modulatory effect on OPN.

Our laboratory, is involved since long in evaluating the effects of a wide spectrum of natural compounds/phytochemicals in the management of various diseases, where one such study carried out and reported earlier was that allicin from garlic and resveratrol from grapes showed equally good effects as denosumab (Prolia®), which recently is being employed in the treatment of osteoporosis by in suppressing OPN levels. Therefore, in the present study, experiments with denosumab (Prolia®) were not carried out, and instead, the already observed results denosumab (Prolia®) as referenced above, were compared with those of punicalagin, quercetin, resveratrol and ascorbic acid on OPN expression.

Thus, in view of the above, one of the most striking findings was that punicalagin followed by resveratrol followed by quercetin = ascorbic acid were equally good as denosumab (Prolia®), which has been employed in recent times in the treatment of osteoporosis, in suppressing OPN levels. Denosumab (Prolia®) offers a new approach in the treatment of osteoporosis. Currently, it is being clinically used for the treatment of osteoporosis and cancer-related bone disorders. Denosumab (Prolia®) decreases bone resorption by inhibiting osteoclast formation, function and survival.

Therefore, in the present study, the high magnitude levels of down-regulation/suppression of OPN which was induced by punicalagin, quercetin, resveratrol and ascorbic acid respectively may probably be by blocking RANKL by these natural antioxidants just like denosumab, and in turn, inhibits osteoclast differentiation, activation and survival, and consequently, in turn, favours bone formation over bone resorption, increasing bone mass and reducing the risk of fractures.
Abstract

Bioinformatics is seen as an emerging field with the potential to significantly improve how drugs are found, brought to the clinical trials and eventually released to the marketplace. The docking analysis of ascorbic acid, quercetin, punicalagin, resveratrol with target proteins glutathione peroxidase and TNF-α was carried by HEX software. Docking allows the scientist to virtually screen a database of compounds and predict the strongest binders based on various scoring functions. The molecules binding to a receptor, inhibit its function, and thus act as drug. The collection of ascorbic acid, quercetin, punicalagin, resveratrol with target proteins glutathione peroxidase and TNF-α were identified via docking and their relative stabilities were evaluated using molecular dynamics and their binding affinities, using free energy simulations. Our docking data show strong binding energies ($\Delta E_{\text{Total}}$) of GPx and TNF-α with all the above selected natural antioxidants/polyphenols, and that, our experimental in vitro data are nearly in synergy with docking data.

Hence, in summary, the present study showing effective regulation of TNF-α, sRANKL, OPN, GPx activities, osteoclast formation and differentiation, etc by punicalagin, quercetin, resveratrol and ascorbic acid appears to provide a valuable tool in probing the control molecular mechanism of osteoporosis. And, and in turn, such natural antioxidants/polyphenols having anti-inflammatory properties may serve as potential adjuncts in the better understanding of osteoporosis and its further management.