Quercetin- A Natural Antioxidant From Onion May Be Beneficial In The Management Of Osteoporosis

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Abstract: Background: Bone is a living rigid connective tissue. The main bone degrading disease is osteoporosis that involves the silent decrease in bone strength, accompanied by fragile bone leading to fractures. ROS and TNF-α levels when starts augmenting each other levels synergistically, leads to an increase in bone markers and accelerated osteoclasts activity and hence osteoporosis. Methods: Effects of quercetin was evaluated in the management of osteoporosis by employing cell culture study, ELISA and determination of GPx activity as well as GSH assay. Elevated levels of ROS were arrested by natural antioxidant quercetin, which in turn controls osteoporosis. Results: We report the augmented levels of TNF-α mRNA expression in osteoporosis patients which are in accordance to our earlier reports. Quercetin decreased osteoclasts differentiation ($p<0.001$). Also, at both the gene and protein levels, the levels of TNF-α were also found to be reduced. The results show the suppression in TNF-α mRNA expression with 20-25μg/ml of quercetin in mononuclear cells of patients with osteoporosis. Similar observations at the protein level substantiated the above data. Furthermore, we observed the antioxidant induced amelioration in GPx activity and GSH levels coupled with down regulation of TNF-α in cells of osteoporotic patients, thereby, exhibiting the possible chemo preventive property of quercetin in osteoporosis. Conclusion: Thus we may hope and conclude that the side effects of various drugs and therapies involved in osteoporosis may be overcome by the use of this natural antioxidant ie. Quercetin. [Faiza I NJIRM 2017; 8(2):110-117]

Key Words: Quercetin, glutathione peroxidise (GPx) activity, GSH, TNF-α, natural antioxidant

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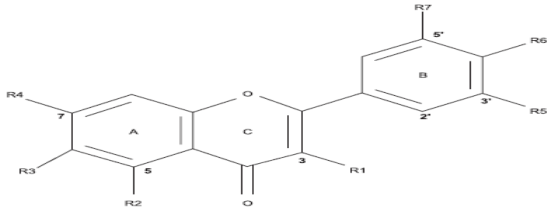
Introduction: Osteoporosis is a silent bone disease as it remains unidentified until a fracture take place.[1] It is mostly after menopause in females due to the deficiency of estrogen hormone.[2] Bone remodelling is a delicate balance between the activities of osteoblasts (bone forming cells) and osteoclasts (bone resorbing cells).[3] Besides many causative factors like age, sex, lifestyle, one of the major causes of this disease is reactive oxygen species (ROS) generated in vivo.

The increased activity of osteoclasts results in the generation of ROS mainly superoxide which then leads to lipid peroxidation and finally to oxidative stress.[4] Besides this, ROS are capable of inducing changes in other bio molecules ie. DNA and protein as well.[5]

Furthermore, TRAP and cathepsin K, expressed by osteoclasts are mainly responsible for bone resorption.[6] ROS is known to destroy the organic matrix of the bone. In recent studies, a global trend has emerged indicating natural compounds as regulators of bone cells in the management of osteoporosis.[7,8]

Investigations carried out recently in various laboratories have revealed that increased consumption of fruits and vegetables having antioxidant character are seen to improve bone condition markedly.[9,10,11] Therefore, antioxidants consumption may prove to be very useful in protecting bones from oxidative damage by scavenging the action of free radicals which are one among the causes of osteoporosis.

Quercetin (3,3',4',5,7-pentahydroxy flavones; mol. wt. 302.24), isolated from onion mainly, is a phytoestrogen,[12] is known to be present in cabbage, garlic, broccoli, tea, blueberries, red wine, apple and grapes[13]. The basic property exerting various beneficial effects is in its anti-oxidant nature. Moreover, being a flavanoid, it scavenges reactive oxygen species (ROS)[14]. The structure of quercetin is shown below.



Next, quercetin has been reported to decrease the damage inflicted by oxidative stress in vitro as well as in vivo in methyl-mercury exposed rats.[15] Also,

quercetin inhibits osteoclasts through its estrogenic ability in long bones of rabbit [16]. Studies[17] have also shown that quercetin at a concentration of 20 µM induced cell death in osteoblastic cell line of mouse via apoptosis in an ERK1/2 dependent manner. However, these results are varying with earlier reports where quercetin does not caused cell death of osteoblastic cells in rat calvaria and also MG-63 human osteoblastic cells.[18,19] Despite positive effects, quercetin have shown to cause DNA damage at a concentration of >30µM and at a concentration exceeding 75 µM it can cause cytotoxic effects in renal cells.[20]

There are 5 hydroxyl groups in quercetin which determines the biological activity and the number of possible derivatives of the compound.[21,22] Glycosides and ethers are the main groups of derivatives of quercetin, where as the sulfate and prenyl are less frequently occurring substituent. Workers have showed that the natural flavanoid quercetin reduces the formation of ROS and thus helps the antioxidant system in maintaining the redox balance in patients.[23]

The objective of this paper was, to observe and evaluate whether the consumption of quercetin, is able to improve antioxidant levels and ultimately helps in the management of osteoporosis through scavenging / regulation of free radicals.

Methods: Study subjects: Prior to any study, Institutional ethical clearance was obtained. Also, prior consent from blood donors was also obtained. Venous blood from healthy volunteers of both sexes (n=30) and blood from patients with osteoporosis (n=30) was obtained from the patients attending J.N. Medical College Hospital of A.M.U. Serum was separated and stored at –20^{o}C until required.

Isolation of peripheral blood mononuclear cells (PBMC) and osteoclastogenesis in culture medium: Ficoll-hypaque density gradient method as described earlier[24,25] was used to isolate PBMCs from both normal healthy volunteers serving as controls (n=30) and osteoporotic patients (n=30).

The PBMCs isolated as stated above from both control and osteoporotic patients were centrifuged at 400g for 30 minutes at 21°C. The buffy layer formed was removed by Pasteur pipette. The remaining cells were washed twice in PBS. Mononuclear cells thus obtained were put to culture at a density of 2×10^{6} cells /cm² on 10 cm petridishes in osteoclastogenic medium as described by us earlier[26,27] as well as by other workers.[28] Thereafter, cells were cultured for 24 hrs, 72 hrs and 120 hrs in osteoclastogenic medium. Next, culture filtrates (CF) were collected and stored at -20^{o}C until required, and the adherent cells were subjected to trypsin digestion for 6 min at 37^{o}C, and subsequently the cells were analysed by TRAP staining.

MTT Cell viability Assay: The adverse effect of varying doses of quercetin (0-100 µg/ml), if any, on adherent cells cultured under osteoclastogenic medium as described above was determined by employing MTT cell viability assay as described by us earlier.[26,27,30,31,32] The percentage of viable cells was calculated by the previously described formula and the results are expressed as “Viable cells (% of control cells)”.[33]

Quantification of TRAP-positive multinucleated cells: Adherent cells were stained for TRAP with a kit (Sigma-Aldrich, USA) on a 96-well culture plate, according to the manufacturer’s guidelines as well as described by us earlier.[26,27]

Glutathione peroxidise (GPx) activity and Glutathione (GSH) assay: Activity of glutathione peroxidase (GPx) was measured as described by us earlier and elsewhere[26,27,30,31,32,34]. Similarly, GSH levels in quercetin treated / untreated cells was measured according to the method described by us earlier and elsewhere.[26,27,30,31,32,35]

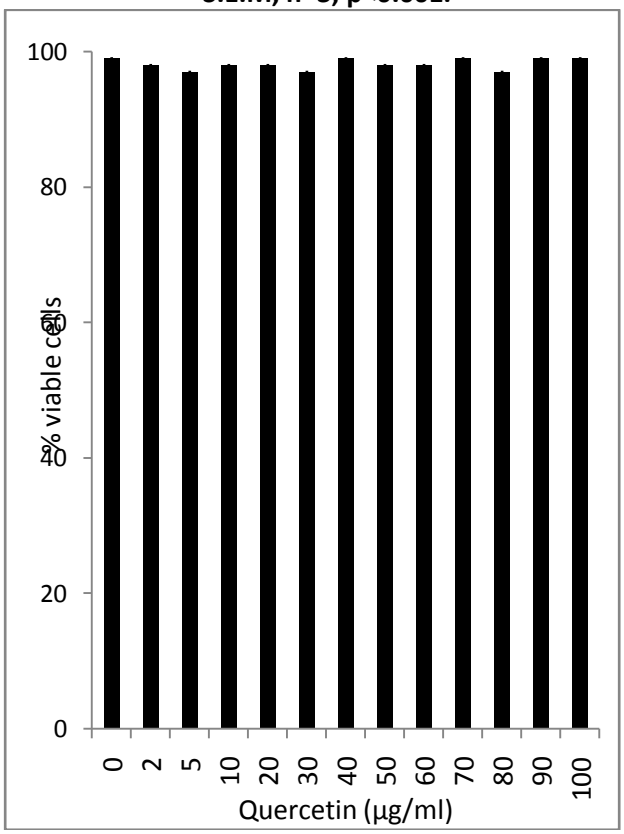
Expression of TNF -α in culture supernatants: The expression of TNF-α in culture supernatants of PBMCs from patients with osteoporosis that were co-cultured with and without quercetin (20µg/ml) was measured by employing the method as described by us earlier.[26,27,30,31,32]

Dose Response Effect of quercetin on generation of osteoclasts from PBMCs: To investigate the effects of quercetin on the generation of osteoclasts from PBMCs, varying doses of quercetin (0-25 µg/ml) were added to the PBMCs cultures , grown at a density of1×10 ^{6}cells/cm² in a 96-well plate, in a osteoclastogeneic culture condition for 1,3 and 5 days in 5% CO_2. The cells were then subjected to TRAP staining as described by us earlier and mentioned above.

Statistical analysis: Paired t test was used for the analysis of the results and are expressed as means ± S.E. of ten experiments. P < 0.05 was considered statistically significant.

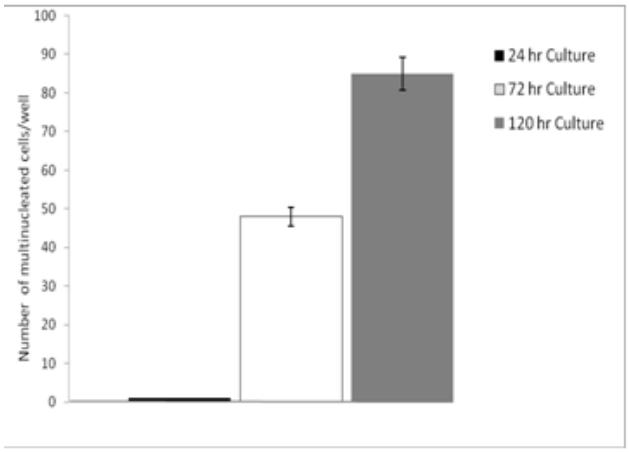
Results: Cell viability: None of the doses (0-100µg/ml) of quercetin exhibited any toxic effects in 24 hr cultures (n=3) (Fig.1). Therefore, 24 hr cultures were safe with the above said doses for further study.

Fig.1. Percent cell viability of monocytes from healthy subjects, co-cultured with varying doses of quercetin for 24 hr. The data represents mean ± S.E.M, n=3; p<0.001.



Generation of osteoclasts precursors in cultures of PBMCs under osteoclastogenic medium: PBMCs were directly taken for the generation of precursors of osteoclasts. It was observed that in day 3 cultures under osteoclatogenic medium, the multinucleated osteoclasts precursors started to appear. Their number was found to increase in culture carried out for 5 days. However, in cultures for the first two days, no appearances of such cells were observed (Fig.2). This is in accordance to our earlier reports. [26,27]

Fig.2. No. of multinucleated cells/well at 24, 72 and 120 hr by TRAP assay. The data represents mean ±S.E.M, n=30; p<0.001.



Dose response of quercetin on the generation of osteoclasts precursors: PBMCs were co-cultured with quercetin in osteoclastogeneic medium for 3 and 5 days. For the selection of dose, a dose response experiment was done, where TRAP assay showed a linear suppression in the production of multinucleated precursor cells. The results showed that there was suppression of around 30-33% in the appearance of osteoclasts precursors in 3 days at a dose of 20-25 µg/ml of quercetin and 45-47% in 5 days cultures at same dose (Fig.3, 4).

Fig.3 TRAP assay showing the dose response effect of quercetin on multinucleated cells in 3 and 5 days cultures. The data represents mean ±S.E.M, n=6; p<0.001.

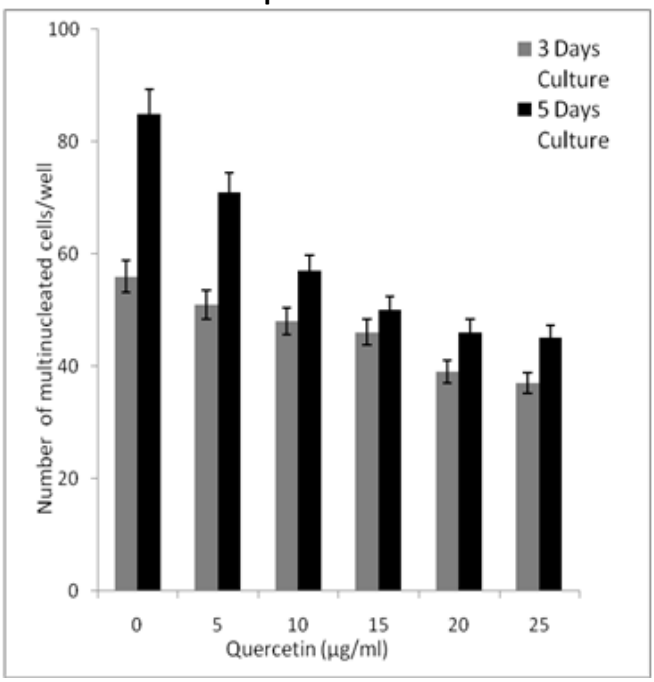
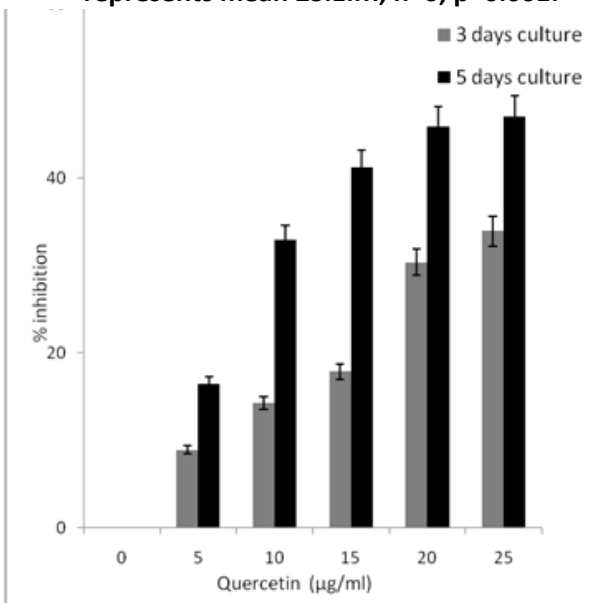


Fig.4. Percent inhibition in multinucleated cells by quercetin in 3 and 5 days cultures. The data represents mean ±S.E.M, n=6; p<0.001.



Evaluation of TNF-α protein expression in 24 hr monocytes culture supernatants: In comparison to healthy controls (n=8), appreciably augmented basal levels of TNF-α were recorded in monocytes culture supernatants of osteoporotic patients (Fig.5). Co-culturing of monocytes of both control and osteoporotic patients with varying doses (0-100 µg/ml) of quercetin showed down regulation of TNF-α secretion in a dose–dependent manner. However, in the healthy controls, low levels of TNF-α remained more or less unaffected at all doses (Fig.6).

Fig.5 TNF-α in 24 hr monocytes culture supernatants of osteoporosis patients compared to healthy controls. The data represents mean ±S.E.M, n=8; p<0.001.

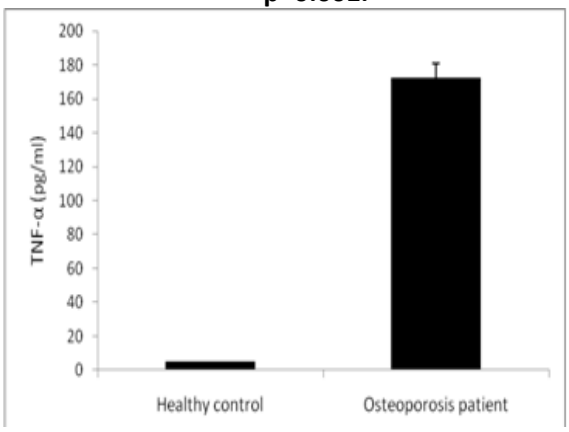
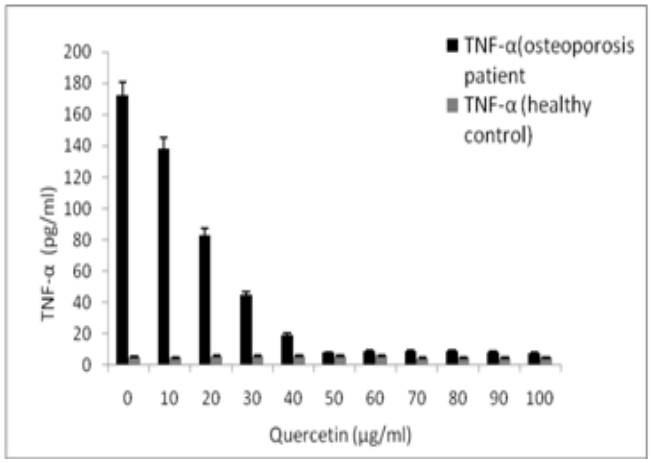


Fig.6. Effect of varying doses of quercetin on the expression of TNF-α in 24 hr monocytes culture supernatants. The data represents mean ±S.E.M, n=6; p<0.001.



Glutathione peroxidase activity: As evident in Fig.7, the GPx activity in osteoporotic patients culture devoid of any quercetin was found to be suppressed appreciably in comparison to healthy controls. Thereafter, monocytes culture supernatants from patients with osteoporosis that were co-cultured with varying doses (0-100 µg/ml) of quercetin revealed GPx activity to be sufficiently/appreciably ameliorated at a dose of around 50-60 µg/ml (Fig.8).

Fig.7. Status of glutathione peroxidase (GPx) activity in 24 hr culture supernatants of monocytes. The data represents mean ±S.E.M, n=20; p<0.001.

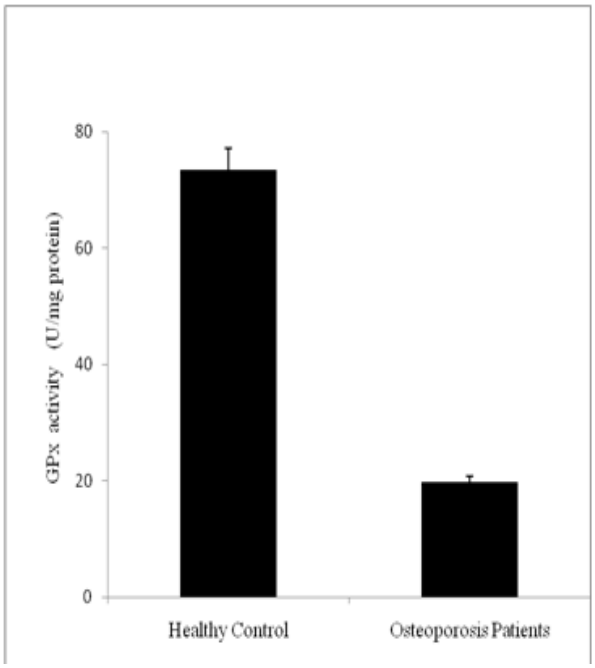
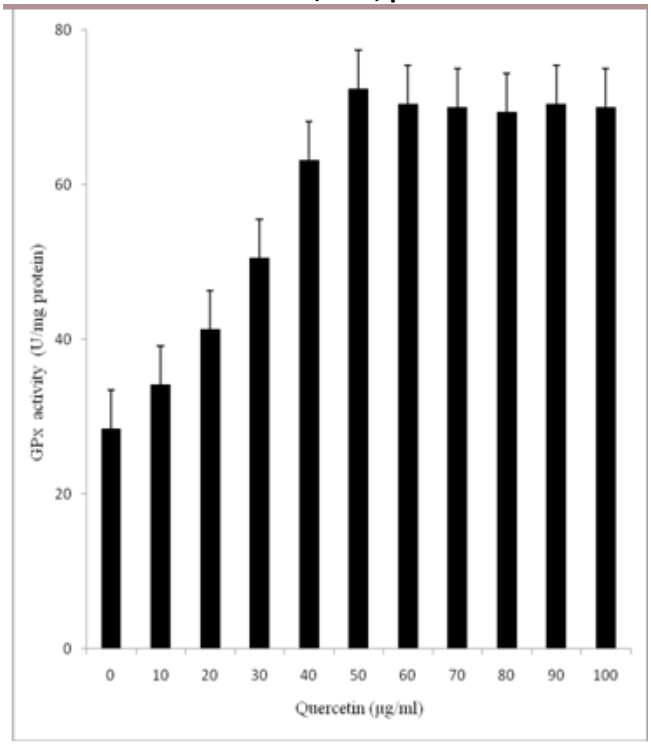


Fig.8. Dose response effect of quercetin on glutathione peroxidase (GPx) activity in 24 hr monocytes culture supernatants. The data represents mean ±S.E.M, n=6; p<0.001.



GSH level: The GSH level was observed to be greatly reduced in osteoporotic patients culture in comparison to controls (Fig.9). With the co-culturing of quercetin, at around 20-25µg/ml the activity was found to be maximally ameliorated / augmented (Fig.10).

Fig.9. Status of intramonocyte glutathione (GSH) levels in healthy and patients monocytes. The data represents mean ±S.E.M, n=20; p<0.001.

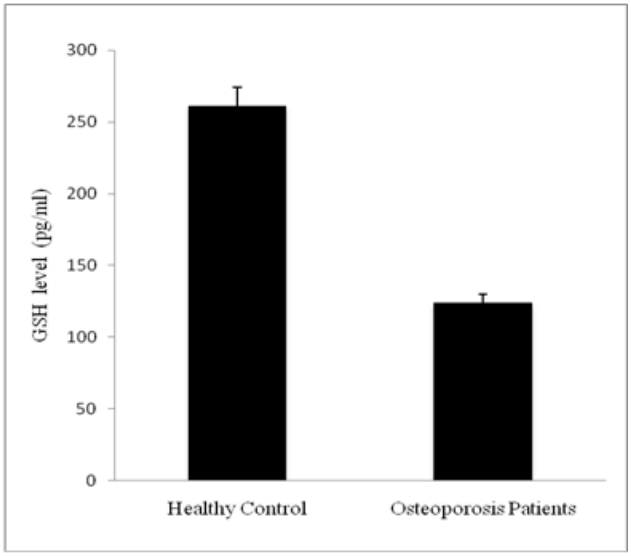
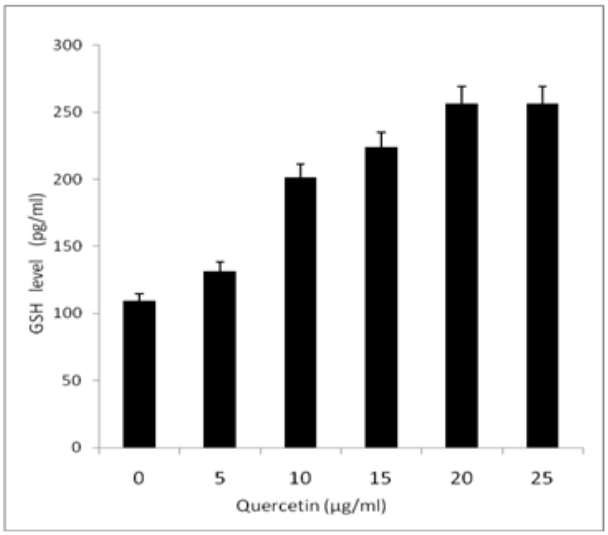


Fig.10. Effect of quercetin on GSH expression in 24 hr monocytes cultures. The data represents mean ±S.E.M, n=6; p<0.001.



Discussion: Osteoporosis is a age related disorder occurring mainly due to the production of ROS leading to oxidative stress.[26,27,36]

ROS have long been associated with disease like atherosclerosis, cancer, diabetes, neurodegenerative diseases, and recently thought to be linked with osteoporosis development,[26,27,37] thereby directing researchers for exploring natural alternatives to combat such diseases.[26,2738]

Naturally occurring antioxidant as well as a flavanoid, namely, quercetin has attained the attention of the researchers due to its high antioxidant status.[39] As evident from our study, quercetin does not have any toxic effects on the viability of cells in 24 hr cultures at concentrations from (0-100µg/ml). But, there was a decline in the viability at or beyond 50µg/ml in 48-120 hr cultures.

Osteoclasts precursors were generated directly from the PBMCs in osteoclastogenic medium. In the first two days there was no appearance of such cells. At day 3, the precursors started to appear, and their number increased at day 5. We report that when quercetin was added to these cultures, the data showed a linear suppression in the production of multinucleated precursor cells at day 3 and day 5. This indicates that quercetin suppresses the generation of osteoclasts precursor.

ROS generated in the cells of osteoporotic patients activates TNF-α, causing further increase in bone loss.[40] Our results showed high levels of TNF-α at the protein level in PBMCs cultured in osteoclastogeneic medium isolated from osteoporotic patients in comparison to the low basal levels found in normal healthy controls. Co-culturing of these PBMCs with varying doses of quercetin was found to down-regulate / suppress the TNF-α level. However controls cells remained unaffected with quercetin treatment. This indicates that quercetin is capable of regulating the TNF-α level via its antioxidant properties through the arrest / scavenging of ROS. The GPx and GSH activities that are markedly decreased in osteoporotic patients in comparison to controls due to increased oxidative stress were also found to be ameliorated with the co-culturing of quercetin. Hence it may have a positive role in the treatment of osteoporosis.

In summary, the recent study has successfully proved that quercetin having antioxidant properties is capable of controlling / inhibiting osteoclasts generation in vitro, and thus, in turn, may act as a valuable tool capable of controlling osteoporosis by scavenging the generation of ROS and inhibiting further degenerative effects. With this, it is hoped that, this natural antioxidant/flavanoid, may be an economical, safe and effective adjunct in the better management of osteoporosis.

Conclusion: Thus, in conclusion, it is hoped that the side effects of various drugs and therapies involved in osteoporosis may possibly be overcome by the use of this natural antioxidant ie. Quercetin, and in turn, may possibly be employed as an adjunct improving the management of osteoporosis.

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