Results
The data of the present study is depicted under four sections as under:

(A) Oxidative Stress Study in Serum and Monocyte Cultures
(B) Immunological study by ELISA
(C) Bone Marker Study by Quantitative Real time RT-PCR
(D) Osteoclast and bone marker study by TRAP and ELISA

In all the sections except section one as mentioned above, we employed allicin-a natural antioxidant from garlic as well as EGCG- a green tea polyphenol, to investigate its regulatory effect on vast parameters as discussed in the results depicted below.

SECTION (A) OXIDATIVE STRESS STUDY IN SERUM AND MONOCYTE CULTURES

(1) GPx activity in serum of osteoporotic patients
Since high oxidative stress is associated with osteoporosis, thus, the anti-oxidant state of osteoporosis patients was assessed by determining the GPx activity in their sera. When compared to healthy subjects, the GPx activity in serum of patients with osteoporosis was appreciably reduced by around 2.2-fold. This is evident from data where GPx activity in sera of osteoporosis patients (n=30) and healthy controls (n=30) was recorded as 38.94 U/ mg protein and 88.12 U/ mg protein respectively (Figure 1). All values represent mean ± SE of ten experiments, p<0.001.

(2) GPx activity in culture supernatants of monocytes
Adherent monocytes obtained from PBMCs of healthy controls as well as patients with osteoporosis were cultured for 24 hours as described in methods. Thereafter, supernatants were analyzed for GPx activity, where patient’s samples exhibited an appreciably suppressed GPx activity (23.25 U/ mg protein) when compared to samples of healthy subjects (69.76 U/ mg protein) (Figure 2). Thus, from the said data, it seems that antioxidant defense system is compromised by around 2.8-fold in patients with osteoporosis. All values are mean ± SE of ten experiments; p<0.001.
FIGURE 1: GPx activity in serum of osteoporosis patients and healthy controls. Data represent mean ± S.E.M. of ten independent experiments. p<0.001 was considered significant.
FIGURE 2: GPx activity in culture supernatants of monocytes of osteoporosis patients and healthy controls. Data represent mean ± S.E.M. of ten independent experiments. p<0.001 was considered significant.
(3) **Determination of intramonocyte glutathione (GSH) levels**

As a matter of fact, glutathione (GSH) being a major tissue thiol antioxidant, an attempt was made to evaluate the intramonocyte levels in 24 hour monocyte cultures of osteoporosis patients as well as in healthy individuals. As depicted in Figure 3, the magnitude of intramonocyte GSH were significantly suppressed in samples of osteoporosis patients (144.32 pg/ml) in comparison to healthy controls (256.65 pg/ml). The data indicates that antioxidant defense gets suppressed/reduced in osteoporosis patients in comparison to non-osteoporosis healthy subjects. All values are mean ± SE of ten experiments; p<0.001.
FIGURE 3: Intramonomocyte Glutathione (GSH) levels in healthy controls and osteoporosis patients. Data represent mean ± S.E.M. of ten independent experiments. p<0.001 was considered significant.
(4) **Dose response effect of allicin from garlic on GPx activity in cultured monocytes supernatants**

Due to the proven beneficial role of allicin from garlic as a natural anti-oxidant in a range of pathological conditions, we opted to study its comparative effect on antioxidant state in supernatants of 24 hours cultured monocytes from patients with osteoporosis and healthy individuals which served as controls.

Monocytes were therefore, co-cultured for 24 hours with varying concentrations of allicin (0, 50, 100, 250, and 500ng/ml). Cultures were harvested and supernatants obtained were subjected to GPx activity determination. Insignificant variation in the activity; 74.17, 76.63, 73.83, 75.38 and 76.27 U/mg protein was recorded for healthy individuals at above mentioned allicin concentrations (Figure 4). On the contrary, in case of osteoporosis patients (Figure 5), the GPx activity was found to increase in a dose-dependent manner from 29.43 U/mg protein, through 36.12, 40.18, 50.08, 67.76 U/ mg protein at 0, 50, 100, 250 and 500 ng/ml allicin. These results clearly prove allicin as an effective natural antioxidant capable of raising the depressed antioxidant state in case of osteoporosis. All values are mean ± SE, p<0.001 and n=6 in each study group.

(5) **Dose response effect of EGCG on GPx activity in monocyte culture supernatants**

Since EGCG from green tea is a known natural antioxidant as well as an immuno-regulator, thus, its effect on GPx activity in monocyte culture supernatants of healthy individuals and osteoporosis patients was also studied for a comparative analysis.

Monocytes were co-cultured for 24 hours with varying doses of EGCG (0, 2, 5, 10, 15 and 20 µg/ml). Supernatants when subjected to GPx activity showed progressive up-regulation in a dose-dependent manner from 33.88 U/mg protein through 40.16, 48.67, 54.97, 70.03 and 74.32 U/ mg protein (Figure 6). On the contrary,
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insignificant variation (71.04, 70.27, 71.40, 75.27, 74.15 and 76.38 U/mg protein) at above mentioned doses of EGCG was recorded in case of healthy individuals (Figure 7). Therefore, EGCG, together with allicin proved to be effective herbal antioxidant in osteoporosis. All values are mean ± SE, p<0.001 and n=6 in each study group.
FIGURE 4: Dose response effect on GPx activity in monocytes cultured supernatants of healthy individuals, treated with Allicin (0-500ng/ml) for 24 hours. Data represent mean ± S.E.M. of six independent experiments. p<0.001 was considered significant.
FIGURE 5: Dose response effect on GPx activity in monocytes cultured supernatants of osteoporosis patients, treated with Allicin (0-500ng/ml) for 24 hours. Data represent mean ± S.E.M. of six independent experiments. p<0.001 was considered significant.
FIGURE 6: Dose response effect on GPx activity in monocytes culture supernatants of osteoporosis patients, treated with EGCG (0-20 µg/ml) for 24 hours. Data represent mean ± S.E.M. of six independent experiments. p<0.001 was considered significant.
FIGURE 7: Dose response effect on GPx activity in monocytes culture supernatants of healthy individuals, treated with EGCG (0-20 μg/ml) for 24 hours. Data represent mean ± S.E.M. of six independent experiments. p<0.001 was considered significant.
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(6) **Dose response effect of allicin on intramonocyte levels of glutathione (GSH)**

Thereafter, an attempt was also made to probe for any regulatory effect of varying doses of allicin (0, 50, 100, 250, and 500 ng/ml) on intramonocyte GSH levels in 24 hours monocyte cultures. Substantially low level (123.22 pg/ml) of intramonocyte GSH was recorded at 0 ng/ml allicin compared to healthy individuals' monocyte cultures (316.28 pg/ml). An appreciable and dose-dependent up-regulation of intramonocyte GSH levels through 169.02, 198.9, 265.2 to 286.2 pg/ml was recorded for osteoporosis patients at 0, 50, 100, 250, and 500 ng/ml allicin (Figure 8). On the contrary, insignificant variation was observed in case of healthy subjects; 315.22, 309.18, 305.12, 303.62 and 305.23 pg/ml (Figure 9). Therefore, allicin efficiently raised the depressed thiol anti-oxidant state in osteoporosis study subjects. All values are mean ± SE, p<0.001 and n=6 in each study group.

(7) **Dose response effect of EGCG from green tea on intramonocyte glutathione (GSH) levels**

Also, effect of varying concentrations of EGCG (0, 2, 5, 10, 15 and 20 μg/ml) on intramonocyte GSH levels in 24 hour monocyte cultures was investigated. Again, significantly reduced levels of intramonocyte GSH were recorded at 0 ng/ml EGCG (118.57 pg/ml) in case of osteoporosis patients when compared to healthy subjects (323.40 pg/ml), which thereafter, increased dose-dependently through 152.10, 168.10, 247.30, 259.43 to 279.65 pg/ml at 2, 5, 10, 15 and 20 μg /ml EGCG respectively (Figure 10). Insignificant variation was observed in case of healthy subjects; 304.70, 297.30, 303.13, 302.23, 299.54 and 301.90 pg/ml at above mentioned doses of EGCG (Figure 11). Thus, EGCG can also be effectively used to improve the degenerating anti-oxidant state in the pathogenesis of osteoporosis. All values are mean ± SE, p<0.001 and n=6.
FIGURE 8: Dose response effect of Allicin (0-500 ng/ml) on Intramonicocyte Glutathione (GSH) levels in 24 hours monocyte cultures of osteoporosis patients. Data represent mean ± S.E.M. of six independent experiments. p<0.001 was considered significant.
FIGURE 9: Dose response effect of Allicin (0-500 ng/ml) on Intramonomocyte Glutathione (GSH) levels in 24 hours monocyte cultures of healthy individuals. Data represent mean ± S.E.M. of six independent experiments. p<0.001 was considered significant.
FIGURE 10: Dose response effect of EGCG (0-20 μg/ml) on Intramonocyte Glutathione (GSH) levels, in 24 hours monocytes culture of osteoporosis patients. Data represent mean ± S.E.M. of six independent experiments. p<0.001 was considered significant.
FIGURE 11: Dose response effect of EGCG (0-20 μg/ml) on Intramonic Glutathione levels, in 24 hours monocytes culture of healthy subjects. Data represent mean ± S.E.M. of six independent experiments. p<0.001 was considered significant.
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(8) Modulation of intramonocyte glutathione (GSH) levels

Intracellular signaling via NFκB is known to be ROS sensitive and given the high oxidative stress in osteoporosis, monocytes from the study groups were 24 hourly co-cultured with 10 mM NAC or 100 μg/ml SN50 or 100 μg/ml SN50/M or 500 ng/ml allicin or 20 μg/ml EGCG for comparative modulation of intramonocyte GSH levels. Control cultures (-) did not receive any treatment and revealed intramonocyte GSH levels of 314.50 and 129.61 pg/ml for osteoporosis and healthy individuals respectively (Figures. 12 and 13 respectively). At 24 hours, NAC, a known anti-oxidant and SN50, an inhibitor of NFκB activation both up-regulated the intramonocyte GSH levels in cultures of osteoporosis patients (243.67 and 269.37 pg/ml respectively). However, SN50/M, an inactive analogue of SN50, at same concentration failed to cause any modulation in osteoporosis patients (139.29 pg/ml). Allicin, a natural anti-oxidant and EGCG, a green tea polyphenol and also an immuno-regulator, was chosen for this modulation study. Interestingly, both allicin and EGCG appreciably up-regulated the intramonocyte GSH levels in osteoporosis patients (279.33 and 258.11 pg/ml respectively). When compared, it was much more potently than either NAC or SN50. Insignificant variation (302.10, 288.70, 304.18, 288.92, 302.40 and 309.30 pg/ml) was recorded in case of healthy individuals with any of the modulating agent used here. These results collectively indicate that the down-regulated intramonocyte GSH levels in osteoporosis patients is NFκB mediated and that allicin and EGCG, both prove as potential natural and safer anti-oxidants in osteoporosis. All values are mean ± SE, p<0.001 and n=6 in each of the study groups.
FIGURE 12: Comparative modulation study of Intramonocyte GSH levels of osteoporosis patients in 24 hour co-culture using NAC, SN50, SN50/M, Allicin and EGCG. (-) denotes no treatment. Data represent mean ± S.E.M. of six independent experiments. p<0.001 was considered significant.
FIGURE 13: Comparative modulation study of Intramonicocyte GSH levels of healthy controls in 24 hour co-culture using NAC, SN50, SN50/M, Allicin and EGCG. (-) denotes no treatment. Data represent mean ± S.E.M. of six independent experiments. p<0.001 was considered significant.
(9) **Determination of serum MDA levels in pre- and post-menopausal patients**

As a step further, following the results showing impaired antioxidant systems in osteoporosis patients as revealed by decreased GPx activity and reduced GSH levels, we measured the levels of a by-product of lipid peroxidation i.e., malondialdehyde (MDA) in the sera of osteoporosis patients to detect further signs of increased oxidative stress. In comparison to healthy group (7.51 ng/ml), the serum MDA values were found to be almost four times higher in osteoporosis patients (28.31 ng/ml) as depicted in Figure 14. All values are mean SE of six experiments i.e., n=6 and p<0.001 in each case.

(10) **Determination of MDA levels in culture supernatants of monocytes**

Adherent monocytes from PBMCs of all the study groups were cultured for 24 hours and supernatants analysed for MDA levels which in osteoporosis patients stood to a near 3.6 times (30.83 ng/ml) the level found in healthy group (9.88 ng/ml) as depicted in Figure 15. Data represent mean SE of six experiments, p<0.001 in each case. Therefore, osteoporotic patients are indeed exposed to high oxidative stress as reflected by high levels of MDA both in serum as well as in supernatants of cultured monocytes.
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FIGURE 14: MDA levels in serum of healthy controls and osteoporosis patients.
Data represent mean ± S.E.M. of six independent experiments. p<0.001 was considered significant.
FIGURE 15: MDA levels in monocyte culture supernatants of healthy individuals and osteoporosis patients. Data represent mean ± S.E.M. of six independent experiments. p<0.001 was considered significant.
(11) **Dose response effect of allicin on MDA levels in supernatants of cultured monocytes**

Next, in order to find out if allicin could prove beneficial in overcoming the augmented oxidative stress so common in osteoporosis, monocytes obtained from the study groups were co-cultured for 24 hours with varying doses of allicin (0-500 ng/ml) and supernatants compared for MDA levels. As is evident from Figure 16, the MDA levels, ranging only from 6.18 ng/ml to 6.99 ng/ml in the supernatants of monocyte cultures of healthy subjects, remained more or less unaltered at all the concentrations of allicin used. While in case of osteoporosis patients, the MDA levels showed a dose-dependent decrease from 26.33 ng/ml when monocytes were cultured alone through 15.61, 13.21, 9.16 to as low as 7.11 ng/ml when treated with 50, 100, 250, and 500 ng/ml allicin respectively (Figure 17). Therefore, at doses of 250 and 500 ng/ml allicin, MDA levels in osteoporosis patients were comparable to those found in healthy subjects thereby upholding potential antioxidant property of allicin in combating high oxidative stress in osteoporosis. All values are mean SE, n=6 in each study group and p<0.001 in each case.

(12) **Dose response effect of EGCG on MDA levels in monocyte culture supernatants**

Apart from the above, monocytes were similarly treated for 24 hours with varying doses of EGCG (0-20 μg/ml) to explore if any positive impact EGCG would show in the pathogenesis of bone loss due to osteoporosis. Thus, the supernatants of the said cultures when analyzed for MDA levels, which showed a progressive down-regulation in osteoporosis patients from 27.93 ng/ml when monocytes were cultured alone and then through 21.62, 17.21, 13.14, 10.11 to as only as 7.93 ng/ml when treated with 2, 5, 10, 15 and 20 μg/ml of EGCG respectively as shown in Figure 18. No significant variation was observed at any of the above mentioned EGCG doses in case of healthy group with MDA levels of 7.09, 7.28, 7.42, 7.39, 7.92, and 7.18 ng/ml respectively (Figure 19). All values are mean SE of n=6 in each study group and p<0.001 in each case. Therefore, EGCG once again proved to be efficient antioxidant in osteoporosis.
FIGURE 16: Dose response effect of Allicin (0-500 ng/ml) on MDA levels in 24 hour monocyte culture supernatants of healthy subjects. Data represent mean ± S.E.M. of six independent experiments. p<0.001 was considered significant.
FIGURE 17: Dose response effect of Allicin (0-500 ng/ml) on MDA levels in 24 hour monocyte culture supernatants of osteoporosis patients. Data represent mean ± S.E.M. of six independent experiments. p<0.001 was considered significant.
FIGURE 18: Dose response effect of EGCG (0-20 μg/ml) on MDA levels in monocyte supernatants of osteoporosis patients, cultured for 24 hours. Data represent mean ± S.E.M. of six independent experiments. p<0.001 was considered significant.
FIGURE 19: Dose response effect of EGCG (0-20 μg/ml) on MDA levels in monocyte supernatants of healthy individuals, cultured for 24 hours. Data represent mean ± S.E.M. of six independent experiments. p<0.001 was considered significant.
SECTION (B) IMMUNOLOGICAL STUDY BY ELISA

(1) Determination of IL-1β levels in sera and supernatants of monocyte cultures of Osteoporosis patients and healthy individuals by ELISA

Presence of high levels of pro-inflammatory cytokine IL-1β in osteoporosis is well established. Thus, an attempt was made to probe the levels of IL-1β in sera as well as monocyte culture supernatants of osteoporosis patients and results compared with those obtained from healthy subjects. ELISA results depicted in Figure 20 clearly show high basal levels of IL-1β in sera as well as monocyte culture supernatants of osteoporosis patients (181.16 pg/ml and 141.36 pg/ml respectively) compared to IL-1β secretion in sera (27.23 pg/ml) and healthy individuals (6.85 pg/ml). All values are mean ± SE; p<0.001 and n=8 in each study group.
FIGURE 20: Levels of IL-1β in serum and monocyte culture supernatants of healthy controls and osteoporosis patients. Data represent mean ± S.E.M. of eight independent experiments. p<0.001 was considered significant.
(2) **Dose-response effect of allicin on expression of IL-1β in monocyte culture supernatants**

Monocytes of study group were cultured for 24 hours with varying concentrations of allicin (0, 50, 100, 250 and 500 ng/ml). Cultures were harvested and supernatants obtained were subjected to ELISA for comparative evaluation of IL-1β secretion. As is evident from Figure 21, the secretion of IL-1β was found to decrease dose-dependently in osteoporosis patients from 161.34 pg/ml through 136.11, 44.12, 30.21, 26.19 pg/ml at 0, 50, 100, 250 and 500 ng/ml allicin respectively. In case of healthy subjects, low IL-1β secretion remained more or less unaffected, which were to the order of 4.51, 4.18, 5.63, 3.56 and 4.25 pg/ml respectively at the above varying doses of allicin (Figure 22). All values are mean ± SE, p<0.001 and n=6 in each of the study groups.

(3) **Dose-response effect of EGCG from green tea on the expression of IL-1β in monocyte culture supernatants**

Thereafter, monocytes of study groups were similarly treated with varying concentrations of EGCG (0, 5, 10, 15 and 20 μg/ml). A similar response was observed with EGCG as was observed above with allicin. In case of osteoporosis patients, IL-1β secretion dose-dependently decreased from 169.12 pg/ml through 144.36, 81.39, 44.26 and 29.11 pg/ml (Figure 23). In healthy individuals, 3.06, 4.35, 3.96, 3.36 and 3.19 pg/ml IL-1β secretion levels were recorded at the respective varying doses of EGCG (Figure 24). All data represent mean ± SE; p<0.001 and n=6 in each study groups.
FIGURE 21: Dose-response effect of varying doses of Allicin (0-500 ng/ml) on expression of IL-1β in 24 hour monocyte culture supernatants of osteoporosis patients. Data represent mean ± S.E.M. of six independent experiments. p<0.001 was considered significant.
FIGURE 22: Dose-response effect of varying doses of Allicin (0-500 ng/ml) on expression of IL-1β in 24 hour monocyte culture supernatants of healthy subjects. Data represent mean ± S.E.M. of six independent experiments. p<0.001 was considered significant.
FIGURE 23: Dose-response effect of varying doses of EGCG (0-20 µg/ml) on expression of IL-1β, in 24 hour culture monocyte culture supernatants of osteoporosis patients. Data represent mean ± S.E.M. of six independent experiments. p<0.001 was considered significant.
FIGURE 24: Dose-response effect of varying doses of EGCG (0-20 μg/ml) on expression of IL-1β, in 24 hour monocyte culture supernatants of healthy subjects. Data represent mean ± S.E.M. of six independent experiments. p<0.001 was considered significant.
Modulation of IL-1β secretion in monocyte culture supernatants

To compare the modulation in IL-1β secretion, monocytes were co-cultured with 10 mM NAC, or 100 μg/ml SN50, or 100 μg/ml SN50/M, or 500 ng/ml allicin, or 20 μg/ml EGCG. Control cultures (-) in each study group did not receive any treatment and revealed IL-1β basal levels of 6.27 and 164.19 pg/ml for healthy subjects (Figure 25), and osteoporosis patients (Figure 26) respectively. At 24 hrs, NAC, a known anti-oxidant and SN50, an inhibitor of NFκB activation both down-regulated the IL-1β secretion in osteoporosis patients (55.14 and 40.61 pg/ml respectively). However, SN50/M, an inactive analogue of SN50 failed to show any modulation in osteoporosis patients (162.32 pg/ml). While allicin, a natural anti-oxidant and EGCG, a green tea polyphenol and also an immuno-regulator, chosen in this modulation study, both produced an appreciable down-regulation of IL-1β secretion in osteoporosis patients (29.16 and 25.22 pg/ml respectively), which was found to be much more effectively than either NAC or SN50. In case of healthy individuals, insignificant variation was observed with any of the modulating agents. These results while indicating that the secretion of IL-1β is NFκB mediated prove efficacy of allicin and EGCG as natural and safer alternative or adjunct as anti-bone resorptive agents. All values are mean ± SE; p<0.001 and n=6 in each study group.
FIGURE 25: Comparative modulation study of levels of IL-1β in healthy subjects, as controls in 24 hour co-culture of monocyte supernatants using NAC, SN50, SN50/M, Allicin and EGCG. (-) denotes no treatment. Data represent mean ± S.E.M. of six independent experiments. p<0.001 was considered significant.
FIGURE 26: Comparative modulation study of levels of IL-1β in osteoporosis patients in 24 hour co-culture of monocyte supernatants, using NAC, SN50, SN50/M, Allicin and EGCG. (-) denotes no treatment. Data represent mean ± S.E.M. of six independent experiments. p<0.001 was considered significant.
SECTION (C) BONE MARKER STUDY BY QUANTITATIVE 'REAL TIME RT-PCR

In this section, attempt has been made for comparative characterization of monocytes at the gene level from osteoporosis patients and compared to those of healthy individuals with respect to bone markers like TNF-α and OPG.

(1) **Expression of Bone Marker TNF-alpha mRNA by real-time RT-PCR**

PBMCs were isolated from the blood of normal healthy individuals and osteoporosis patients to obtain adherent monocytes as described in methods. Monocytes from osteoporosis patients were then subjected to TNF-α mRNA evaluation by real-time RT-PCR and compared with the values of TNF-α mRNA copy number recorded in monocytes from healthy subjects. As is evident from Figure 27, in comparison to healthy subjects’ monocytes, those from osteoporosis patients revealed the presence of high basal levels of TNF-α mRNA copy number which was to the order of 7.16E+08 (p<0.001) These data, therefore, revealed significantly high basal levels of TNF-α mRNA in the monocytes of osteoporosis patients. All values are mean ± SE of six experiments in each study group.
FIGURE 27: Real Time RT-PCR for TNF-α mRNA expression in monocyte cultures of healthy individuals and osteoporosis patients. Expression of TNF-α was corrected to host 18S rRNA and expressed as copies of TNF-α in $10^{10}$ copies of R18 (equivalent to $1 \times 10^6$ cells). Data represent mean ± S.E.M. of six independent experiments. $p<0.001$ was considered significant.
(2) **Expression of Bone Marker OPG mRNA by real-time RT-PCR**

Next, monocytes from the study group were subjected to OPG mRNA evaluation by real-time RT-PCR. In case of osteoporosis patients, the data obtained revealed higher basal levels of OPG mRNA copy number which was ~ 8.3 logs (p<0.001) the healthy subjects level (Figure 28). This result therefore, indicated higher basal level expression of OPG mRNA in osteoporosis patient’s PBMCs.
FIGURE 28: Real Time RT-PCR for OPG mRNA expression in monocyte cultures of healthy individuals and osteoporosis patients. Expression of OPG was corrected to host 18S rRNA and expressed as copies of OPG in $10^{10}$ copies of R18 (equivalent to $1 \times 10^6$ cells). Data represent mean ± S.E.M. of six independent experiments. $p<0.001$ was considered significant.
(3) Dose response effect of allicin from garlic and EGCG from green tea on human house keeping gene R18

In view of proven health benefits of garlic since ancient times, we chose here to study action of allicin, an active component of garlic and also EGCG, the biologically active compound of green tea, on monocytes isolated from PBMCs of the study groups. First, an attempt was made to probe the effect of allicin as well as EGCG, if any, on the human house keeping gene R18 whereby it was observed that neither allicin (0-500 ng/ml) nor EGCG (0-20 μg/ml), at any of their respective concentrations when these were used to co-culture the monocytes, had any significant effect on the expression of human house keeping gene R18 as revealed by quantitative real-time RT-PCR (Figure 29 and 30 respectively for allicin and EGCG).
FIGURE 29: Real Time RT-PCR for dose response effect of Allicin on the expression of human house keeping gene R18. Data represent mean ± S.E.M. of six independent experiments. $p<0.001$ was considered significant.
FIGURE 30: Real Time RT-PCR for dose response effect of EGCG on the expression of human house keeping gene R18. Data represent mean ± S.E.M. of six independent experiments. p<0.001 was considered significant.
(4) **Dose response effect of allicin on TNF-α expression**

Here, we first started with allicin to investigate its action upon TNF-α mRNA gene expression for which purpose adherent monocytes from PBMCs of osteoporosis patients and healthy subjects were co-cultured with varying concentrations of allicin (0-500 ng/ml) for 24 hours. Cultures devoid of allicin i.e., at 0 ng/ml served as control. Monocytes were then subjected to TNF-α mRNA evaluation by real-time RT-PCR. It is evident from Figure 31, the expression of TNF-α mRNA was dose-dependent showing significant downregulation in its copy number in case of osteoporosis patients from 7.14E+08 in untreated monocytes through ~3.8 logs, ~4.9 logs to ~5.8 logs when monocytes were treated with 50, 100, 250, and 500 ng/ml allicin respectively. However, no significant variation was observed in case of healthy individuals whereby expression tended to ~0.05 logs, 0.6 logs, 0.6 logs and 0.7 logs of that observed in untreated monocytes. The data in this experiment prove allicin as an effective natural suppressor of augmented TNF-α mRNA levels in osteoporosis whereby it can be used as a potent anti bone-resorptive agent in this kind of bone pathogenesis. Data represent mean ± SE of six experiments in each study group i.e., n=6 and p<0.001 in each case.

(5) **Dose response effect of allicin on OPG mRNA expression**

Allicin was also used to check its effect on OPG mRNA expression. Thus, adherent monocytes from PBMCs of osteoporosis patients and healthy subjects were co-cultured with various doses of allicin (0-500 ng/ml) for 24 hours. Monocytes were subjected to OPG mRNA evaluation by real-time RT-PCR and results depicted in Figure 32. As evident, the expression of OPG mRNA showed dose-dependent downregulation with increasing doses of allicin. In case of osteoporosis patients, the OPG mRNA copy number was recorded as 3.64E+09 when monocytes were cultured alone. Thereafter it showed a downregulation of as much as 0.6 logs, ~4 logs, ~5.6 logs and ~6.3 logs when monocytes were cultured with 50, 100, 250, and 500 ng/ml allicin respectively.
No significant change with these concentrations was observed in case of healthy individuals whereby expression tended to 0.05 logs, 0.07 logs, 0.09 logs and 0.15 logs starting from 2.66E+00 (untreated monocytes). Data represent mean ± SE of six experiments (n=6) in each study group and p<0.001.
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FIGURE 31: Real Time RT PCR for dose response effect of Allicin (0-500 ng/ml) on the expression of TNF-α mRNA from PBMCs of healthy individuals and osteoporosis patients, cultured for 24 hours. Cultures receiving 0 ng/ml allicin served as controls. Expression of TNF-α was corrected to host 18S rRNA and expressed as copies of TNF-α in $10^{10}$ copies of R18 (equivalent to $1 \times 10^6$ cells). Data represent mean ± S.E.M. of six independent experiments. $p<0.001$ was considered significant.
(6) **FIGURE 32:** Real Time RT PCR for dose response effect of Allicin (0-500 ng/ml) on OPG mRNA expression from PBMCs of osteoporosis patients and healthy subjects, cultured for 24 hours. Cultures receiving 0 ng/ml allicin served as controls. Expression of TNF-α was corrected to host 18S rRNA and expressed as copies of TNF-α in 10^{10} copies of R18 (equivalent to 1 \times 10^6 cells). Data represent mean ± S.E.M. of six independent experiments. p<0.001 was considered significant.
(7) **Dose response effect of EGCG on TNF-α expression**

Thereafter, it was also attempted to probe the effect of varying concentrations of EGCG (0-20 μg/ml) on the expression of TNF-α mRNA in 24 hour cultures of PBMCs isolated from osteoporosis patients and healthy subjects. As is evident in Figure 33, monocytes when subjected to TNF-α mRNA evaluation by real-time RT-PCR showed dose-dependence on EGCG in all study groups. In case of osteoporosis patients, significant downregulation of TNF-α mRNA copy number of as much as 0.22 logs, 3.33 logs, ~ 5.4 logs, 5.6 logs and 5.95 logs was observed in monocytes treated with 2, 5, 10, 15 and 20 μg/ml of EGCG respectively when compared to untreated monocytes.

On the contrary, no significant change in gene expression (0.08 logs, 1.03 logs, 1.17 logs, 1.19 and 1.24 logs compared to untreated monocytes) was observed in case of healthy subjects at any of the concentrations of EGCG used. The data in this result point to EGCG especially at 10, 15 and 20 μg/ml doses as effective natural immunoregulator that can be used against the pathogenesis of bone loss in osteoporosis. All values are mean ± SE of six experiments (n=6) (p<0.001).

(8) **Dose response effect of EGCG on OPG mRNA expression**

Thereafter, change in the expression of OPG mRNA with EGCG was also probed, whereby varying doses of EGCG (0-20 μg/ml) were used to co-culture the monocytes isolated from PBMCs of osteoporosis patients and healthy subjects for 24 hours. Monocytes were then subjected to OPG mRNA evaluation by real-time RT-PCR, wherein the results are depicted in Figure 34. As evident, the expression of OPG mRNA showed dose-dependent downregulation with increasing doses of EGCG. In case of osteoporosis patients, the OPG mRNA copy number was recorded as 3.05E+09 when monocytes were cultured alone, thereafter a downregulation of as much as ~ 0.53 logs, 2.04 logs, ~ 3.5 logs, 4.4 logs and 5.3 logs was recorded at 2, 5, 10, 15 and 20 μg/ml EGCG co-culture respectively. Insignificant variation in gene expression in case of healthy subjects from 1.94E+00
at 0 µg/ml EGCG, through 0.035 logs, 0.063 logs, 0.077 logs, 0.069 logs to 0.089 logs was observed at these doses of EGCG respectively. These results indicated that EGCG significantly down regulated the OPG mRNA expression in osteoporosis patients. All data are mean ± SE of six experiments (p<0.001).
FIGURE 33: Real time RT PCR for dose response effect of EGCG (0-20 µg/ml) on TNF-α mRNA expression, from PBMCs of healthy individuals and osteoporosis patients, cultured for 24 hours. Cultures receiving 0 µg/ml EGCG served as controls. Expression of TNF-α was corrected to host 18S rRNA and expressed as copies of TNF-α in $10^{10}$ copies of R18 (equivalent to $1 \times 10^6$ cells). Data represent mean ± S.E.M. of six independent experiments. $p<0.001$ was considered significant.
FIGURE 34: Real time RT PCR for dose response effect of EGCG (0-20 μg/ml) on OPG mRNA expression, from PBMCs of healthy individuals and osteoporosis patients, cultured for 24 hours. Cultures receiving 0 μg/ml EGCG served as controls. Expression of OPG was corrected to host 18S RNA and expressed as copies of OPG in $10^{10}$ copies of R18 (equivalent to $1 \times 10^6$ cells). Data represent mean ± S.E.M. of six independent experiments. p<0.001 was considered significant.
(9) **Effect of 10mM NAC on TNF-α and OPG mRNA expression**

Next, we used N-acetyl cysteine (NAC) which is a known anti-oxidant to effect the change in expression of TNF-α and OPG mRNA extracted from monocyte fraction of peripheral blood collected from osteoporosis patients. This was done in order to compare the effects as revealed by allicin and EGCG in our study on the expression of TNF-α and OPG mRNA in osteoporosis patient monocytes. Therefore, monocytes were either treated or untreated with 10 mM NAC for 24 hours and then subjected to evaluation of TNF-α and OPG mRNA by real-time RT-PCR. As is evident in Figure 35, untreated monocytes revealed higher expression of both TNF-α mRNA (~ 7.8 logs) and OPG mRNA (~ 8.4 logs) in osteoporosis patients while treatment with 10 mM NAC caused a downregulation of ~ 4.6 logs in TNF-α mRNA level and 4.2 logs in OPG mRNA expression level in this study group. While in previous results, allicin and EGCG have revealed more potent effect on both the genes expression. Data in this figure are mean ± SE of six individual experiments; p<0.001.
FIGURE 35: Real time RT PCR for effect of 10 mM NAC on TNF-α mRNA and OPG mRNA expression, in monocyte cultures of osteoporosis patients, cultured for 24 hours. Expression of TNF-α and OPG was corrected to host 18S rRNA and expressed as copies of TNF-α and OPG in $10^{10}$ copies of R18 (equivalent to $1 \times 10^6$ cells). Data represent mean ± S.E.M. of six independent experiments. p<0.001 was considered significant.
SECTION (D) OSTEOCLAST AND BONE MARKER STUDY
BY TRAP AND ELISA

(1) Generation of Human Osteoclast Precursors from Peripheral Blood Mononuclear Cells (PBMCs)

Peripheral blood mononuclear cells (PBMCs) were used directly for the generation of osteoclast precursors after centrifugation with Ficoll-Hypaque. After the 3 day culture duration in osteoclastogenic medium (α-MEM culture medium supplemented with 10% FCS, 100U/ml penicillin, 100 μg/ml streptomycin, 50ng/ml M-CSF and 25ng/ml RANKL), multinucleated osteoclast precursors were observed to appear and the number increased after 5 days of culture, as revealed by Tartrate Resistant Acid Phosphatase (TRAP) staining (Figures 36(a,b,c) and 37(a,b,c)). However, there was no appearance of osteoclast precursors after 24 h (1 day) of culture (data not shown). The number of multinucleated preosteoclasts, arising from PBMCs isolated from the blood of normal healthy individual (data not shown) and osteoporotic patients (Figure 36, 37), were counted by TRAP staining. Interestingly, we observed an individual variation in osteoclast generation from different donors as depicted by different number of multinucleated cells in Figure 36 and Figure 37.
FIGURE 36 (a): Individual variation in generation of human osteoclast precursors from PBMCs of osteoporosis patients (1 to 10), cultured for 3 days in osteoclastogenic medium, quantified as TRAP (+ve) multinucleated cells as assessed by TRAP staining. The results are shown as means ± SEM.
FIGURE 36 (b) : Individual variation in generation of human osteoclast precursors from PBMCs of osteoporosis patients (11 to 20), cultured for 3 days in osteoclastogenic medium, quantified as TRAP (+ve) multinucleated cells as assessed by TRAP staining. The results are shown as means ± SEM.
FIGURE 36 (c) : Individual variation in generation of human osteoclast precursors from PBMCs of osteoporosis patients (21 to 30), cultured for 3 days in osteoclastogenic medium, quantified as TRAP (+ve) multinucleated cells as assessed by TRAP staining. The results are shown as means ± SEM.
FIGURE 37 (a): Individual variation in generation of human osteoclast precursors from PBMCs of osteoporosis patients (1 to 10), cultured for 5 days in osteoclastogenic medium, quantified as TRAP (+ve) multinucleated cells as assessed by TRAP staining. The results are shown as means ± SEM.
FIGURE 37 (b): Individual variation in generation of human osteoclast precursors from PBMCs of osteoporosis patients (11 to 20), cultured for 5 days in osteoclastogenic medium, quantified as TRAP (+ve) multinucleated cells as assessed by TRAP staining. The results are shown as means ± SEM.
FIGURE 37 (c): Individual variation in generation of human osteoclast precursors from PBMCs, of osteoporosis patients (21 to 30), cultured for 5 days in osteoclastogenic medium, quantified as TRAP (+ve) multinucleated cells as assessed by TRAP staining. The results are shown as means ± SEM.
(2) Effect of Epigallocatechin gallate (EGCG) and Allicin on the Generation of Human Osteoclasts

Interestingly, we observed that co-culturing of PBMCs with EGCG (20 µg/ml) or Allicin (500 ng/ml) in osteoclastogenic medium for 3 and 5 days resulted in an appreciable amount of reduction in appearance of multinucleated osteoclast precursors (Figure 38 {a,b,c} and 39 {a,b,c} for EGCG and Figures 40 {a,b,c} and Figures 41 {a,b,c} for Allicin respectively). Hence, this reflects the potential of EGCG and allicin to exert regulatory effect in osteoclast generation and differentiation. The above dose of EGCG and Allicin was selected after performing dose response experiment, where TRAP assay revealed a linear suppression in the formation of multinucleated cells was observed (Figure 42 and Figure 43 respectively). Nearly 20-25% suppression in appearance of multinucleated cells was observed in cultures receiving 15 µg/ml and 20 µg/ml of EGCG relative to control cultures devoid of any EGCG (Figure 42). Interestingly, around 30-35% suppression in appearance of multinucleated cells was observed in cultures receiving 250 ng/ml and 500 ng/ml of allicin relative to control cultures devoid of any allicin (Figure 43).
FIGURE 38 (a): Dose dependant reduction in appearance of multinucleated osteoclast precursors, from different donors (osteoporosis patient number 1 to 10), co-cultured with and without EGCG (20µg/ml) in osteoclastogenic medium for 3 days. The results are shown as means ± SEM.
FIGURE 38 (b): Dose dependant reduction in appearance of multinucleated osteoclast precursors, from different donors (osteoarthritis patient number 11 to 20), co-cultured with and without EGCG (20μg/ml) in osteoclastogenic medium for 3 days. The results are shown as means ± SEM.
FIGURE 38 (c): Dose dependant reduction in appearance of multinucleated osteoclast precursors, from different donors (osteoporosis patient number 21 to 30), co-cultured with and without EGCG (20µg/ml) in osteoclastogenic medium for 3 days. The results are shown as means ± SEM.
FIGURE 39 (a): Dose dependant reduction in appearance of multinucleated osteoclast precursors, from different donors (osteoporosis patient number 1 to 10), co-cultured with and without EGCG (20µg/ml) in osteoclastogenic medium for 5 days. The results are shown as means ± SEM.
FIGURE 39 (b): Dose dependant reduction in appearance of multinucleated osteoclast precursors, from different donors (osteoporosis patient number 11 to 20), co-cultured with and without EGCG (20µg/ml) in osteoclastogenic medium for 5 days. The results are shown as means ± SEM.
FIGURE 39 (c): Dose dependant reduction in appearance of multinucleated osteoclast precursors, from different donors (osteoporosis patient number 21 to 30), co-cultured with and without EGCG (20μg/ml) in osteoclastogenic medium for 5 days. The results are shown as means ± SEM.
FIGURE 40 (a): Dose dependant reduction in appearance of multinucleated osteoclast precursors, from different donors (osteoporosis patient number 1 to 10), co-cultured with and without, Allicin (500 ng/ml) in osteoclastogenic medium for 3 days. The results are shown as means ± SEM.
FIGURE 40 (b): Dose dependant reduction in appearance of multinucleated osteoclast precursors, from different donors (osteoporosis patient number 11 to 20), co-cultured with and without, Allicin (500 ng/ml) in osteoclastogenic medium for 3 days. The results are shown as means ± SEM.
FIGURE 40 (c): Dose dependant reduction in appearance of multinucleated osteoclast precursors, from different donors (osteoporosis patient number 21 to 30), co-cultured with and without, Allicin (500 ng/ml) in osteoclastogenic medium for 3 days. The results are shown as means ± SEM.
FIGURE 41 (a): Dose dependant reduction in appearance of multinucleated osteoclast precursors, from different donors (osteoporosis patient number 1 to 10), co-cultured with and without, Allicin (500 ng/ml) in osteoclastogenic medium for 5 days. The results are shown as means ± SEM.
FIGURE 41 (b): Dose dependant reduction in appearance of multinucleated osteoclast precursors, from different donors (osteoporosis patient number 11 to 20), co-cultured with and without, Allicin (500 ng/ml) in osteoclastogenic medium for 5 days. The results are shown as means ± SEM.
FIGURE 41 (c): Dose dependant reduction in appearance of multinucleated osteoclast precursors, from different donors (osteoporosis patient number 21 to 30), co-cultured with and without, Allicin (500 ng/ml) in osteoclastogenic medium for 5 days. The results are shown as means ± SEM.
FIGURE 42: Dose dependant suppression in appearance of multinucleated cells treated with EGCG (0-20 μg/ml) as assessed by TRAP assay. NT denotes no treatment (Control). Data represent mean ± S.E.M. of three independent experiments. p<0.001 was considered significant.
FIGURE 43: Dose dependant suppression in appearance of multinucleated cells treated with Allicin (0-500 ng/ml) as assessed by TRAP assay. NT denotes no treatment (Control). Data represent mean ± S.E.M. of three independent experiments. $p<0.001$ was considered significant.
(3) Determination of Human sRANKL levels in culture supernatants of healthy controls and osteoporosis patients

Apart from the above, an attempt was also made to probe the levels sRANKL in culture supernatants of healthy controls (n=6) and osteoporosis patients (n=6) by ELISA. In comparison to healthy controls (P<0.01), patient’s cultures exhibited around 9-fold augmented levels of sRANKL (pg/ml; P<0.001) (Figure 44).
FIGURE 44: Levels of Human sRANKL in culture supernatants of healthy controls and osteoporosis patients as depicted by ELISA. Data represent mean ± S.E.M. of six independent experiments. p<0.001 was considered significant.
(4) **Dose response effect of allicin on sRANKL levels in culture supernatants of healthy controls and osteoporosis patients**

Monocytes of study groups were treated with varying concentrations of Allicin (0, 50, 100, 250 and 500 ng/ml). In case of osteoporosis patients, sRANKL secretion dose-dependently decreased from 30.68 pg/ml at through 25.36, 18.12, 9.56 and 4.01 pg/ml with 50, 100, 250 and 500 ng/ml allicin respectively (Figure 45; P<0.001). On the contrary, healthy controls exhibited in between 1.33 – 3.2 pg/ml of RANKL (data not sure). Next, after dose response evaluation, an attempt was also made to re-check the data by co-culturing with the maximum dose of allicin (500 ng/ml) selected in the study, and that, similar results were observed to the one’s observed above in dose response experiments at the maximum dose (Figure 46; P<0.001). All data represent mean ± SE; p<0.001 and n=6 in each study groups.

(5) **Computational evaluation of allicin-induced percent suppression in the secretion of sRANKL in culture supernatants of osteoporosis patients**

Thus, computation of the data revealed that allicin down regulated / suppressed the secretion of sRANKL by around 17.34%, 40.93%, 68.83% and 86.92% with 50, 100, 250 and 500 ng/ml allicin respectively (Figure 47). The IC_{50} was computed out to be in between 100 -125 ng/ml.
FIGURE 45: Dose response effect of Allicin (0-500 ng/ml) on sRANKL levels in culture supernatants of osteoporosis patients. No or negligible effect was observed in healthy controls (data not shown). Data represent mean ± S.E.M. of six independent experiments. p<0.001 was considered significant.
FIGURE 46: Level of Human sRANKL in monocyte culture supernatants of various healthy individuals and osteoporosis patients that were co-cultured with the maximum dose (500ng/ml) of Allicin for five days. Data represent mean ± S.E.M. of six independent experiments. p<0.001 was considered significant.
FIGURE 47: Computational evaluation of Allicin-induced percent suppression in the secretion of sRANKL in culture supernatants of osteoporosis patients. The results are shown as means ± SEM.
(6) **Dose response effect of EGCG on sRANKL levels in culture supernatants of healthy controls and osteoporosis patients**

Similarly, monocytes of study groups were treated with varying concentrations of EGCG (0, 2, 5, 10, 15 and 20 µg/ml). In case of osteoporosis patients, sRANKL secretion was found to dose-dependently decrease from 32.65 pg/ml at through 27.81, 22.45, 17.29, 13.07 and 9.44 pg/ml with 2, 5, 10, 15 and 20 µg/ml EGCG respectively (Figure 48; \( P<0.001 \)). On the contrary, healthy controls exhibited in between 1.3 – 3.2 pg/ml of RANKL (data not sure). Next, after dose response evaluation, an attempt was also made to re-check the data by co-culturing with the maximum dose of EGCG (20 µg/ml) selected in the study, and that, similar results were observed to the one’s observed above in dose response experiments at the maximum dose (Figure 49; \( P<0.001 \)). All data represent mean ± SE; \( p<0.001 \) and \( n=6 \) in each study groups.

(7) **Computational evaluation of EGCG-induced percent suppression in the secretion of sRANKL in culture supernatants of osteoporosis patients**

Computational analysis of the data revealed that EGCG down regulated / suppressed the secretion of sRANKL by around 14.82%, 31.24%, 47.04%, 59.96% and 71.08% with 2, 5, 10, 15 and 20 µg/ml EGCG respectively (Figure 50). The IC\(_{50}\) was computed out to be in between 7.5 – 10 µg/ml of EGCG.
FIGURE 48: Dose response effect of EGCG (0-20µg/ml) on sRANKL levels in culture supernatants of osteoporosis patients. No or negligible effect was observed in healthy controls (data not shown). Data represent mean ± S.E.M. of six independent experiments. p<0.001 was considered significant.
FIGURE 49: Level of Human sRANKL in monocyte culture supernatants of various healthy individuals and osteoporosis patients that were co-cultured with the maximum dose (20μg/ml) of EGCG for five days. Data represent mean ± S.E.M. of six independent experiments. p<0.001 was considered significant.
FIGURE 50: Computational evaluation of EGCG-induced percent suppression in the secretion of sRANKL in culture supernatants of osteoporosis patients. The results are shown as means ± SEM.
Next, an attempt was also made to carry out modulation of sRANKL in the presence or absence of TNF-alpha (2 ng/ml) along with allicin (500 ng/ml) or EGCG (20 μg/ml). Results show that there was negligible effect of TNF-alpha alone or with a combination of both TNF-alpha and allicin on healthy control cultures in comparison to untreated cultures (Figure 51). On the contrary, patient’s samples / cultures exhibited sRANKL levels to the order of 31.39 pg/ml (P<0.001), which in turn was found to be augmented to 43.24 pg/ml (P<0.001) in patient cultures receiving 2 ng/ml TNF-alpha (Figure 51). Interestingly, patient’s cultures receiving a combination of both TNF-alpha (2 ng/ml) and allicin (500 ng/ml) exhibited an appreciable down-regulation / suppression in the secretion of sRANKL (12.28 pg/ml; P<0.001) (Figure 51).

Similarly, an attempt was also made to carry out modulation of sRANKL in the presence or absence of TNF-alpha (2 ng/ml) along with EGCG (20 μg/ml). Results show that there was negligible effect of TNF-alpha alone or with a combination of both TNF-alpha and EGCG on healthy control cultures in comparison to untreated cultures (Figure 52). On the contrary, patient’s samples / cultures exhibited sRANKL levels to the order of 31.39 pg/ml (P<0.001), which in turn was found to be augmented to 43.24 pg/ml (P<0.001) in patient cultures receiving 2 ng/ml TNF-alpha (Figure 52). Interestingly, patient’s cultures receiving a combination of both TNF-alpha (2 ng/ml) and EGCG (20 μg/ml) exhibited an appreciable down-regulation / suppression in the secretion of sRANKL (17.54 pg/ml; P<0.001) (Figure 52). Thus, in the above depicted modulation study wherein TNF-alpha was co-cultured along with either allicin or EGCG, computational analysis of the data revealed that allicin inhibited the secretion of sRANKL by around 60% whereas EGCG inhibited the same by around 44%.
FIGURE 51: Level of human sRANKL in culture supernatants of monocyte cultures of osteoporosis patients that were co-cultured with or without 2 ng/ml of TNF-α or with a combination of 2 ng/ml TNF-α and 500 ng/ml of Allicin for five days. p<0.001 was considered significant.
FIGURE 52: Level of Human sRANKL in culture supernatants of monocyte cultures of osteoporosis patients that were co-cultured with or without 2 ng/ml of TNF-α or with a combination of 2 ng/ml TNF-α and 20 μg/ml of EGCG for five days. *p<0.001* was considered significant.
(9) Visualization of multinucleated cells / osteoclasts by TRAP assay

Monocyte cultures from healthy control as well as that of osteoporosis patient were co-cultured without or with 500 ng/ml of allicin and 20 μg/ml of EGCG respectively for 5 days. Similarly, one of the cultures from patient received 2 ng/ml TNF-alpha. The detection of TRAP activity was performed using test kits from Sigma according to the manufacturer's instructions as described in methods. As evident from Figure 53, healthy control monocyte cultures did not show any multinucleated cells/osteoclasts, whereas those from osteoporosis patient exhibited appreciable number of multinucleated cells/osteoclasts (Figure 54). Interestingly, monocyte cultures of 5 days for osteoporosis patient that were co-cultured with 2 ng/ml of TNF-alpha exhibited an enhanced number of multinucleated cells/osteoclasts (Figure 55), when compared to cultures devoid of any TNF-alpha as was evident from the above said Figure 54.

Next, in monocyte cultures of osteoporosis patient, when co-cultured with 500 ng/ml of allicin and 20 μg/ml of EGCG respectively for 5 days the TRAP assay data showed that both 500 ng/ml allicin (Figure 56) as well as 20 μg/ml EGCG (Figure 57), inhibited / suppressed / down-regulated the formation of multinucleated cells/osteoclasts. The suppression was clearly more in cultures receiving allicin in comparison to EGCG.
FIGURE 53: Normal healthy human monocytes were cultured for 5 days as described in methods. No multinucleated cells were detected.
FIGURE 54: Human monocytes from osteoporosis patients were cultured for 5 days as described in methods, and thereafter subjected to TRAP staining. appreciable numbers of multinucleated cells were observed.
FIGURE 55: Human monocytes from osteoporosis patients were co-cultured with 2 ng/ml TNF-alpha for 5 days as described in methods, and thereafter subjected to TRAP staining. An enhanced number of multinucleated cells were observed in comparison to cultures devoid of TNF-alpha.
FIGURE 56: Human monocytes from osteoporosis patients were co-cultured with 500 ng/ml allicin for 5 days as described in methods, and thereafter subjected to TRAP staining. An appreciably reduced number of multinucleated cells were observed in comparison to cultures devoid of allicin.
FIGURE 57: Human monocytes from osteoporosis patients were co-cultured with 20 μg/ml EGCG for 5 days as described in methods, and thereafter subjected to TRAP staining. An appreciably reduced number of multinucleated cells were observed in comparison to cultures devoid of EGCG.