Chapter 5

Conclusion
CONCLUSION

In conclusion, the following can be inferred from the present study that:

1. In comparison to healthy controls, high magnitude suppression in GPx activity and intramonomocyte GSH levels were observed in monocytes cultures of osteoporosis patients.

2. Punicalagin, resveratrol, quercetin and ascorbic acid dose-dependently ameliorated the suppressed GPx activity and GSH levels in osteoporosis patients.

3. Reactive oxygen species (ROS) mediated activation of monocytes of osteoporosis patients resulted in the induction of enhanced/augmented basal levels of TNF-α and IL-1β.

4. Punicalagin, resveratrol, quercetin and ascorbic acid efficiently induced down-regulation in TNF-α and IL-1β in monocytes cultures of osteoporosis patients.

5. A dose in between 0 – 100 µg/ml of punicalagin or resveratrol or quercetin or ascorbic acid failed to show any adverse/toxic effect in 24 hours cultures under osteoclastogenic medium. 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. Interestingly, 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.

6. All the four natural antioxidants/polyphenols namely punicalagin, resveratrol, quercetin and ascorbic acid that were separately co-cultured with PBMCs in osteoclastogenic medium for 3 and 5 days resulted in an appreciable amount of suppression/down-regulation/reduction in appearance of multinucleated osteoclast precursors. This in turn, reflects upon the beneficial potential of punicalagin, resveratrol, quercetin and ascorbic acid to exert regulatory effects in osteoclast generation and differentiation.
7. In comparison to healthy controls, cultures of osteoporosis patients exhibited high magnitude of augmented levels of sRANKL and osteopontin.

8. Punicalagin, resveratrol, quercetin and ascorbic acid which are having antioxidant as well as anti-inflammatory properties, dose-dependently suppressed/decreased sRANKL and osteopontin secretions in cultures of osteoporosis patients.

9. All the natural antioxidants/polyphenols employed in this study exhibited potential antioxidant as well as anti-bone resorptive properties. This was inferred from results observed regarding suppression/down-regulation of bone markers like sRANKL, osteopontin, TNF-α and IL-1β.

10. Modulation with calcitonin (positive modulator), failed to overcome or minimize the potent negative modulatory effects of punicalagin, resveratrol, quercetin and ascorbic on osteopontin.

11. *In vitro* culture studies showed that out of these four natural antioxidants, punicalagin proved to be the most potent suppressor/down-regulator of OPN and sRANKL, followed by resveratrol and in turn, followed by quercetin = ascorbic acid.

12. One of the most striking findings was that when data of punicalagin, resveratrol, quercetin and ascorbic acid was compared to the data of denosumab (Prolia) obtained previously by our lab (Hasan, PhD Thesis, 2014) it was found that all the four above said natural antioxidants were equally good as denosumab.

13. Docking data revealed strong binding of all the four natural antioxidants/polyphenols selected in the present study i.e. punicalagin, resveratrol, quercetin and ascorbic with glutathione peroxidase (GPx) and TNF-α.

Thus, based on the results of the present work, it is hoped that the natural antioxidants employed here and the findings so obtained would prove to be of great help both economically and in terms of safety in the management and treatment of osteoporosis.