Summary and Conclusion
Summary

The present study was carried out to explore the comparative efficacy of EGCG Vs nano EGCG to abate atherosclerosis in aorta of high cholesterol diet fed rats.

- EGCG was successfully synthesised by iono tropic gelation method
- The synthesised EGCG nanoparticle was characterized and confirmed as nanoparticle based on size, shape and drug encapsulation by PSA, SEM, AFM and FTIR.
- Rats administered with high cholesterol diet showed elevated levels of total cholesterol triglyceride, LDL cholesterol and VLDL cholesterol along with concomitant decrease in HDL cholesterol, which was effectively alleviated by EGCG and nano EGCG supplementation there by decreasing the risk of atherosclerosis. This authenticate that hypocholesterolemic activity of EGCG was preserved to a greater extent in the nanoparticle.
- High cholesterol diet supplementation resulted in intimal thickening and presence of necrotic core. Supplementation with EGCG and nano EGCG have reduced intimal thickening and no sign of necrotic core was observed, other than mild cellular vacuolization which was confirmed by H&E staining of aorta section.
- Increased free radical generation and macromolecular damage was observed during HCD feeding. Supplementation of EGCG and nano EGCG has effectively prevented molecular damage caused by free radicals.
- Both enzymatic and non-enzymatic antioxidants were decreased significantly in HCD fed rats. EGCG and nano EGCG supplementation has significantly ameliorated the antioxidant defense system.
HCD feeding have not only increased LDL levels, but also increased levels of oxLDL in serum of rats. Consequently the inflammatory cytokines TNF-α, IL-1β and IL-6 were also increased in serum of HCD fed rats. Supplementation of EGCG has brought back these levels to near normal in treated rats.

There was an increased expression of inflammatory markers such as TNF-α, IL-1β, IL-6, MCP-1 and NF-κB in the aorta of rats supplemented with HCD. Supplementation of EGCG and nano EGCG have abated their levels, implicating the anti-inflammatory activity of EGCG have and nano EGCG.

HCD fed rats showed marked increase in the levels of cell adhesion molecules (VCAM-1 and P-Selectin) in the aorta. Supplementation of EGCG and nano EGCG has decreased the levels of cell adhesion molecules to a significant extent, which helps in reducing monocyte infiltration.

Increased expression of MMP-2 and MMP-9 in HCD was observed expression in aorta of HCD fed rats, whose levels were positively regulated by inflammatory cytokines. Supplementation of EGCG and nano EGCG have normalised their levels.

In vitro analysis of oxLDL induced inflammatory cytokine via NF-κB pathway revealed that oxLDL increased the TNF-α, IL-1β and IL-6 secretion by increasing NF-κB nuclear translocation. Treatment with EGCG and nano EGCG resulted in decreased NF-κB nuclear translocation, which in turn reduced TNF-α, IL-1β and IL-6 release from macrophage.

In vitro analysis of foam cell formation has revealed that oxLDL elicited transformation of macrophage IC-21 cells to foam cells. Whereas cell treated with EGCG and nano EGCG have abated lipid uptake to a greater extent.
EGCG and nano EGCG have prevented IC-21 macrophage cell line from oxLDL induce apoptosis, which was confirmed by flow cytometry analysis.

Docking of EGCG with NF-κB with DNA in its bound and unbound form has shown extensive interaction between them.

**Conclusion**

The present study was designed to investigate the comparative efficacy of EGCG before and after encapsulation as nanoparticle with chitosan as the vehicle. Lipid profile analysis demonstrated that a lower concentration of nano EGCG (4mg/kg body weight) could bring the same effect as that of EGCG at a higher concentration (100mg/kg of body weight) indicating its increased bioavailability and cost effectiveness. EGCG and nano EGCG reduced the levels of oxidative stress marker, oxLDL, oxidative signalling mediated proinflammatory cytokines and chemokine expression by suppressing the NF-κB pathway. In vitro cell culture work using IC-21 macrophages demonstrated that EGCG inhibits NF-κB translocation into the nucleus. The data of NF-κB EGCG docking using *in silico* tools hints that EGCG might bind to NF-κB bound with or without DNA with extensive interactions. EGCG binding site overlaps with the DNA binding site. However its mechanism to prevent NF-κB translocation is yet to be explored.