Summary & Conclusion
11.1 Summary
The results obtained from different parameters studied to investigate the regulation of hyperalgesia by ROS and natural antioxidants curcumin and resveratrol are summarized as below-

1. Decrease in paw withdrawal latency (PWL) was observed at different time points 2h, 6h, 12h, 24h, 48h in CFA induced rats. Curcumin treatment caused significant increase in PWL indicating its anti-hyperalgesic activity.

2. Elevated ROS level was observed in paw skin and spinal cord (L4-L6) after 6h of CFA injection, which was reduced in both tissues by curcumin treatment. The level of ROS was higher in paw skin in comparison to spinal cord.

3. Differential effect on antioxidant enzymes was observed in paw skin and spinal cord. The activities of catalase, SOD, GPx and GR were decreased in paw skin and increased in spinal cord by CFA induction at 6h. Opposite response of antioxidant enzymes in paw skin and spinal cord might be related to difference in ROS level.

4. Curcumin treatment brought the activities of antioxidant enzymes towards normal in both the tissues.

5. Level of pro-inflammatory cytokines was increased in paw skin, whereas the changes were insignificant in spinal cord.

6. Curcumin treatment brought the level of cytokines in paw skin towards normal, although it was ineffective in spinal cord.

7. Resveratrol showed anti-hyperalgesic response in PWL test at different time points 2h, 6h, 12h, 24h, 48h in CFA induced rats as shown by curcumin. PWL was brought up to normal in late phase (48h).

8. ROS level was differentially modulated during hyperalgesia in tissue specific manner. In paw skin, the elevated level of ROS in early phase (6h) was further increased in late phase (48h). Whereas in spinal cord, ROS level was increased in early phase and remained constant up to late phase. Resveratrol treatment could decrease the ROS level in both tissues and both phases.

9. The activities of antioxidant enzymes were decreased in paw skin and increased in spinal cord during early phase in hyperalgesic rats. However, activities of these enzymes remained unchanged during late phase, except catalase which showed elevated activity in
spinal cord as found in early phase. Resveratrol treatment of hyperalgesic rats brought back these changes towards normal level.

10. Levels of pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) were enhanced in CFA induced hyperalgesia at peripheral level (paw skin). Resveratrol treatment did not show any effect.

11. CFA induced hyperalgesia was maintained by time specific (late phase) secretion of pro-inflammatory cytokines at the central level (spinal cord), which was decreased by resveratrol treatment.

12. Decline in the elevated level of COX-2 in CFA induced rats was observed after resveratrol treatment in both phases and at both levels. However, the role of iNOS in hyperalgesia and its regulation by resveratrol was limited to peripheral level.

13. Level of pERK was increased in spinal cord of hyperalgesic rats in both phases and was decreased by resveratrol. Expression of COX-2 showed a correlation with modulation of ERK signaling by resveratrol.

14. PWL test in H₂O₂ induced hyperalgesic rats showed significant increase in thermal hyperalgesia at different time points 20min, 2h, 6h, 24h. However TNF-α level and paw edema was significantly increased only after 2h, indicating that H₂O₂ induced thermal hyperalgesia was developed prior to inflammation and oxidative stress.

15. H₂O₂ administration induced ERK phosphorylation in both A and C nociceptive fibers.

16. H₂O₂ induction caused an increase in the expression of TNFR1 in nociceptive neurons which was co-localized with pERK.

17. Pre-treatment with SFK inhibitor (PP1) and MEK inhibitor (PD98059) decreased the level of pERK in DRG as well as thermal hyperalgesia, highlighting the role of SFK mediated ERK signaling in H₂O₂ induced hyperalgesia.

18. Pre-treatment with PTP inhibitor (sodium orthovanadate) also diminished hyperalgesia, although the pERK level was further increased. Discrepancy in molecular and behavioral data suggests differential role of PTP in DRG and spinal cord.

19. Combination of MEK, SFK and PTP inhibitors did not exhibit synergistic anti-hyperalgesic effect, which indicates their involvement in hyperalgesia in a linear pathway of signaling.
20. ERK signaling in CFA induced hyperalgesia followed similar mechanism as that of \( \text{H}_2\text{O}_2 \) induced hyperalgesia. CFA induced thermal hyperalgesia involved SFK mediated ERK phosphorylation in DRG.

21. CFA induced ERK signaling leads to increased expression of TNFR1 in DRG which may participate in peripheral sensitization of nociceptive neurons.

11.2 Conclusion

Delineated signaling mechanism during CFA induced inflammatory hyperalgesia indicates that ROS is involved in sensitization of nociceptors via SFK mediated ERK activation. Natural antioxidants curcumin and resveratrol possess anti-hyperalgesic activity via modulation of oxidative status by decreasing ROS level, as well as modulation of pro-oxidant and antioxidant mediators.