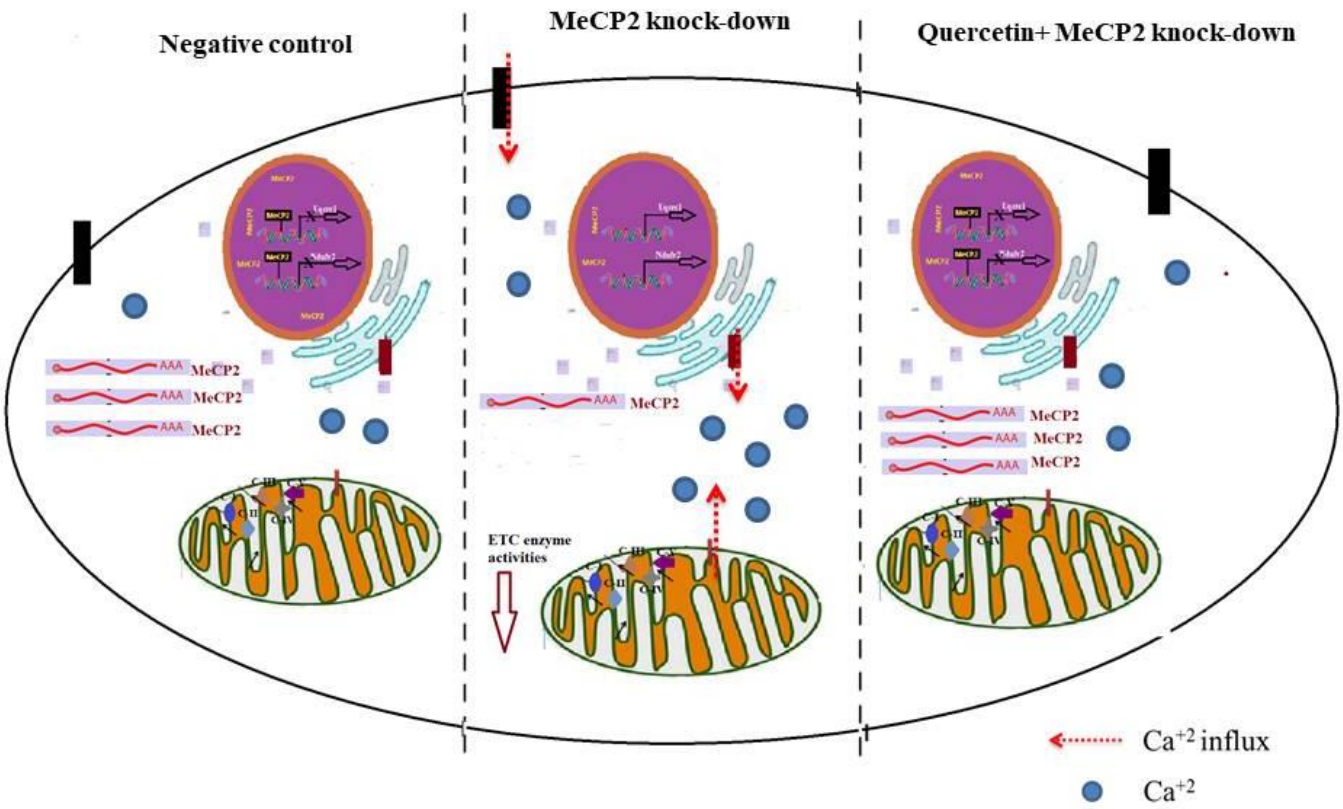
Chapter-VI

Overall summary & conclusion

Objective 1: To determine the effects of quercetin on mitochondrial dysfunction mediated by MeCP2 deficiency in astrocytes.

- Previous reports have shown mitochondrial abnormalities in MeCP2 null mouse models and patients with MeCP2 mutation (Kriaucionis et al. 2006, Gold et al. 2014, Valenti et al. 2017, Shulyakova et al. 2017).
- Increased Uqcrc1 and Ndufv2 genes expression with minor up-regulated Ndufv2 protein expression in MeCP2 knock-down astrocytes was observed.
- Enzyme activities were significantly reduced in MeCP2 knock-down astrocytes.
- Increased intracellular calcium and decreased mitochondrial membrane potential were found in MeCP2 knock-down astrocytes.
- Quercetin normalized MRC genes expression but increased MRC enzyme activities compare to MeCP2 knock down alone were observed that indicates quercetin's multiple targets to ameliorate the imbalance in context-dependent manner.
- Quercetin normalized intracellular calcium and mitochondrial membrane potential in MeCP2 knock-down astrocytes.
- In C6 glial cells, modulated intracellular calcium and ROS were observed in quercetin treated MeCP2 knock-down cells.
- As a whole, the results confirm mitochondrial dysfunction in MeCP2-deficient astrocytes and positive implication of quercetin in restoring the function.

Fig. 6.1 Diagrammatical illustration of Chapter-3 result summary

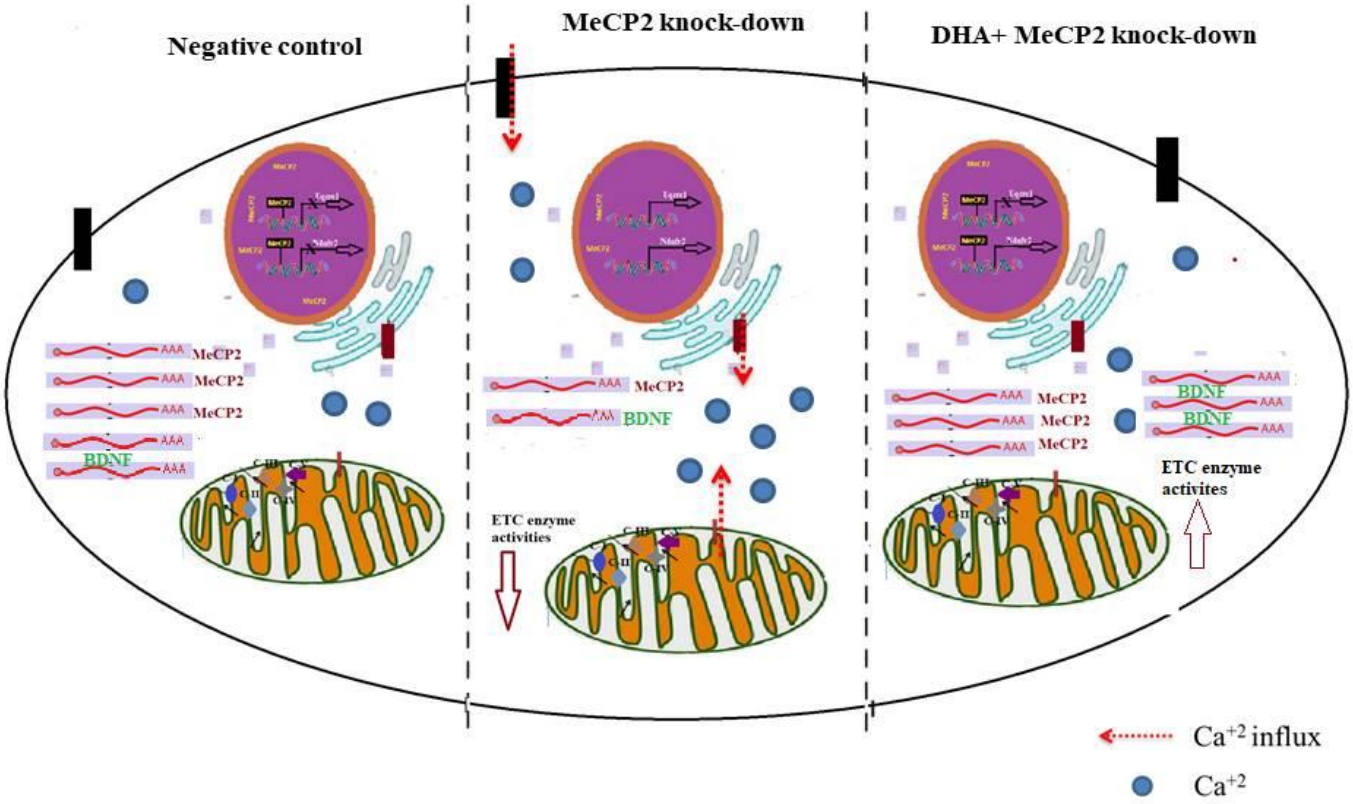


Objective 2: To determine the effects of DHA on mitochondrial dysfunction mediated by MeCP2 deficiency in astrocytes.

- DHA normalized Uqcrc1 but did not alter Ndufv2 gene expression significantly.
- It also increased MeCP2 and BDNF genes expression in MeCP2 knock-down astrocytes.
- The western blot analysis showed increased MeCP2 and Ndufv2 proteins expressions in DHA treated MeCP2 knock-down astrocytes.
- In Spectrophotometric analysis of mitochondrial respiratory complexes also the enzyme activities were found to be increased in DHA treated MeCP2 knock-down astrocytes as compared to MeCP2 knock-down cells in a dose dependent manner.
- Also, the cytosolic calcium was restored in DHA treated MeCP2 knock-down cells.
- In spite of these alterations, ROS and mitochondrial membrane potential did not alter significantly in DHA treated MeCP2 knock-down astrocytes.
- In C6 glial cells, modulated intracellular calcium and ROS were observed in DHA treated MeCP2 knock-down cells.

- Overall, the data indicates multiple target site and roles of DHA at micromolar (25,100µM) concentrations.

Fig. 6.2 Diagrammatical illustration of Chapter-4 result summary

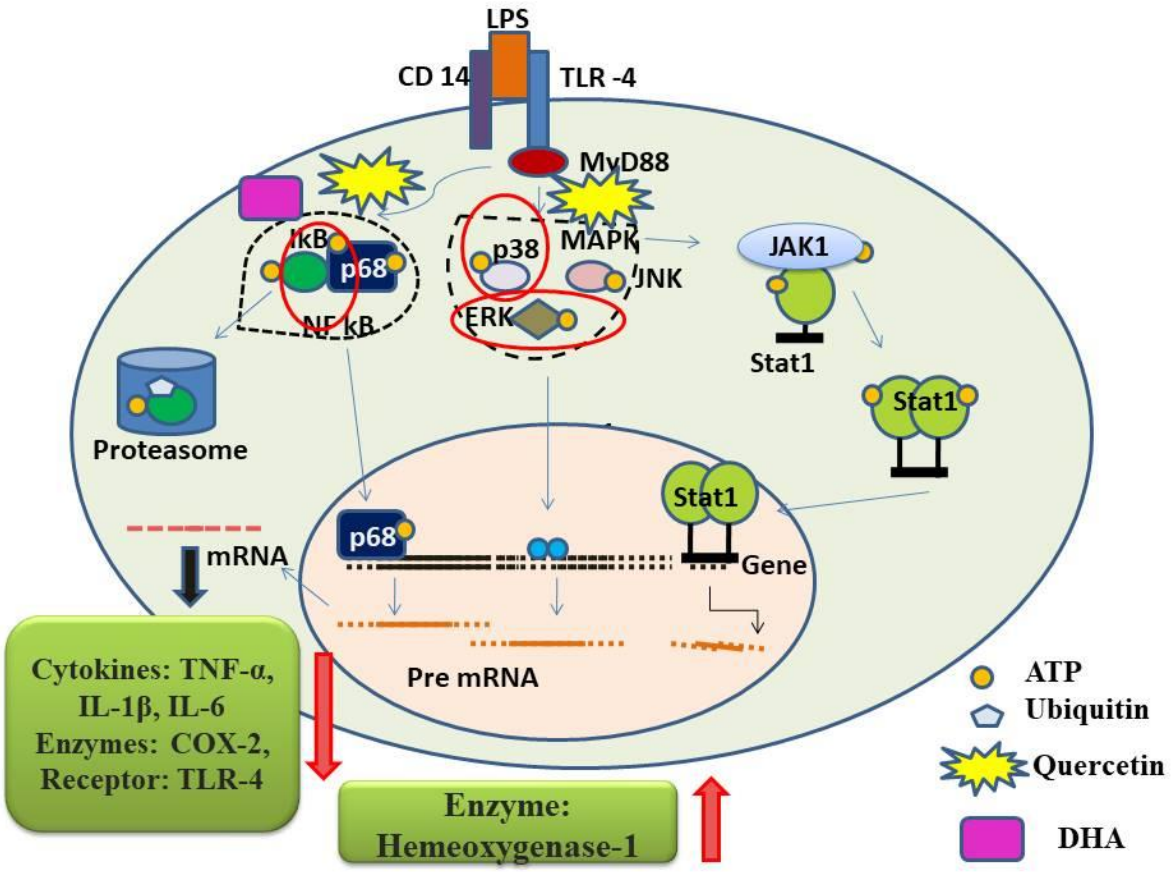


Objective 3: To evaluate anti-inflammatory role of quercetin and DHA on LPS activated astrocytes.

- LPS alone did not induce ligand dependent TLR-4 mRNA level alteration in astrocytes.
- 1µg/ml LPS caused significant up regulation of pro-inflammatory molecules-IL-1β, IL-6, TNF-α and COX-2 in a time dependent manner.
- LPS incubation for 8 hrs was found to be the optimal time point to observe the elevated gene expression of above mentioned pro-inflammatory markers.
- Data show that quercetin hydrate (100µM and 50µM) down regulates the IL-1β, IL-6, TNF-α, COX-2 and up regulates HO-1 genes expression in a dose dependent manner.

- Quercetin significantly decreased phospho p38 and p-IκB-α proteins but increased pERK1/2 protein level in dose dependent manner
- DHA treatment reduced IL-1β, COX-2 and TLR-4 mRNA levels and increased HO-1 mRNA level in LPS activated astrocytes.
- pERK1/2 level was more increased after 30min LPS exposure in DHA pre-incubated astrocytes as compared to LPS alone.

Fig. 6.3 Diagrammatical illustration of Chapter-5 result summary



Though the beneficial effects of quercetin are evident in various cell types and *in vivo* due to its abilility to traverse through BBB owing to P-glycoprotein transporters and lipophilicity (Faria et al. 2010, Youdim et al. 2004),the limiting factors of quercetin for therapeutics are: (i) Quercetin aglycones have tendency to conjugate in enterocytes and BBB with methylates, Glucuronates or sulfates (Kroon et al. 2004) (ii) it causes renal toxicity at higher doses (Ferry et al. 1996) and (iii)

Lipophilicity and affinity for intestinal efflux pumps i.e. P-glycoprotein, MRP2 and cytochrome P450 (Penalva et al. 2017). The doses taken into present *in vitro* study are supra-physiological and not dietary relevant, in spite of that fact, our idea for studying these concentrations was to check if the higher concentrations can ameliorate inflammation in astrocytes. The next step would be to achieve these concentrations in brain by establishing the drug delivery system en route directly to the brain. One of such method is intranasal transport which is the direct transport from the nasal cavity to the brain (Van Woensel et al. 2013). The understanding of these doses *in vivo* through direct delivery to brain would nullify the arguments on its availability to the brain.