Synopsis of the thesis on

ROLE OF FLAVONOID AND POLYUNSATURATED FATTY ACID IN GLIAL CELL FUNCTIONS AND NEUROINFLAMMATION

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Introduction:

The central nervous system possesses two major types of cells: Neurons and Glia. In humans, neurons and glia are roughly equal in number (Azevedo et al., 2009) and are interdependent in functions (Barres, 2008). There are 4 types of glial cells in CNS viz. oligodendrocytes, astrocytes, microglia and ependymal cells.

Among all, Astrocytes possess wide spectrum of functions from cradle to grave of CNS such as guiding development of neurons and oligodendrocytes by releasing neurotrophic factors, contribute to the metabolism of neurotransmitters, store glycogen (energy source), provide nutrient support, and so on. (Montgomery, 1994; Wang and Bordey, 2008)

Astrocytes make very important contributions to CNS metabolism. They take up glucose from blood vessels and furnish energy metabolites to cells in grey and white matters. Mitochondrion is the central organelle for metabolism as it contains enzymes for energy molecule- ATP production. In the CNS diseases, mitochondrial dysfunction plays a very critical role (Keane et al., 2011).Mitochondrial dysfunction through glutathione depletion, glutamate excitotoxicity, altered gene expression of electron transport chain complexes, abnormal Ca^{+2} elevation, oxidative stress, decreased ATP levels are possible underlying mechanisms (Pieczenik & Neustadt, 2007; Streck et al., 2014).Recently it has also been reported that Astrocytes donate healthy mitochondria to neurons after stroke (Hayakawa et al., 2016) thus mitochondrial dysfunction in Astrocytes makes neurons also vulnerable to cell death (Volobouéva et al., 2007).

Methyl-CpG-binding protein 2 (MECP2) is a global transcriptional factor, known to regulate wide array of genes positively and negatively depending on the genome context in which it is operated. The electron transport chain (ETC) is coded by over 850 nuclear DNA (nDNA) and 37 mitochondrial DNA (mtDNA) genes. Recent reports provide an insight into mitochondrial dysfunction by altering electron transport chain complexes genes expressions and enzyme activities in brain of MeCP2 null mouse (Kriaucionis et al., 2006) and RETT syndrome patients (Pecorelli et al., 2013). However, whether MeCP2 altered expression causing change in electron transport chain complexes activities in turn at what extent affects glial cell functions is still not much clear. Cell mitochondria also function as calcium store and release calcium into cytosol according to the stimulus which further regulates calcium dependent kinases and calcium signalling pathways. There are growing body of evidences that suggest role of MeCP2 in calcium homeostasis in neurons (Mironov et al., 2009; Marchetto et al., 2010). Cytosolic calcium was found to be higher in resting state preBötC respiratory neurons in $MeCP2^{-/y}$ mouse model of Rett syndrome (Mironov et al., 2009). Electron transport chain and mitochondrial calcium signalling are interconnected and impairment in any might affect the other. (Nicholls, 2005)

Astrocytes also act as a bridge between nervous and immune system by expressing receptors involved in innate immunity including toll like receptors (TLR-2,TLR-4), components of complement system, and MHC-II,B7, CD-40 receptors which are critical in T-cell activation. Moreover, they release pro-inflammatory and anti-inflammatory cytokines and chemokines that act as immune mediators in cooperation with microglia and provoke adaptive immune system (Dong and Benveniste, 2001; Falsig et al., 2006). Increased activation of pro-inflammatory cytokines are reported to have deleterious effects in neuroinflammatory diseases such as Multiple sclerosis, Parkinson's disease, Alzheimer's disease, and AIDS dementia (Allan and Rothwell, 2003; Maragakis and Rothstein, 2006; Sharma et al., 2010; Li et al., 2011). Astrocytes bring about release of proinflammatory mediators that not only exert paracrine effects in neighbouring cells, but also promote autocrine effects thus grounds higher astroglial reactivity in response to inflammation (Shih et al., 2006). Astrocytes also undergo reactive astrogliosis, a hallmark of many neurodegenerative diseases which involves proliferation, morphological changes and enhanced glial fibrillary acidic protein (GFAP) expression. Although, astrogliosis is beneficial as it leads to the increased production of growth factors and neurotrophins that supports neuronal survival and promotes neuronal growth, but on the other hand it forms glial scars ultimately which is detrimental for neuronal function (Hatten et al., 1991; Sofroniew and Vinters, 2010). Thus, the astrocytes have the potential to impact both beneficially and detrimentally on surrounding neural and non-neural cells. Thus, overall, mitochondrial impairment and inflammation in astrocytes are two of many manifestations for causing central nervous system diseases.

Currently steroidal and non-steroidal drugs are given to patients with such diseases but these conventional drugs have not been successful to cure chronic inflammatory disorders and long term administration has adverse effects.

Quercetin, a plant flavonol, is documented to have anti-oxidant (Jackson et al., 2006; Cevik et al., 2013; Song et al., 2013),anti-inflammatory (Shen et al., 2002; Cho et al., 2003, Jackson et al., 2006, Al-Fayez et al., 2006,Bhaskar et al., 2011, Zhang et al, 2011), cell protective (Cao et al., 2007; Silva et al., 2008; Yousef et al., 2010, Schültke et al., 2010,Wang et al., 2011), anti-proliferative (Spencer et al., 2003), proliferative and regenerative (Dihal et al., 2006; Wang et al., 2011; Wu et al., 2014) roles in various neuronal and non-neuronal cell types. It can traverse through BBB owing to P-glycoprotein transporters and lipophilicity.(Spencer, 2008). It is effective in improving mitochondrial dysfunctions in Huntington's disease model (Sandhir et al., 2013). It has also been found to modulate L-type calcium channel in Pituitary Tumor (GH3) cells and Neuronal NG108-15 cells differentially that indicates the ability of Quercetin in regulating ion channels dependent on cell type or calcium level(Wu et al.,2003). Interestingly, brain derived neurotrophic factor (BDNF) has been found to be improving calcium regulation speculated to be mediated by SERCA, Ca^{2+}

ATPase that transfers Ca^{2+} from the cytosol of the cell to the lumen, in MeCP2 deficient neurons (Mironov et al., 2009). Quercetin has been documented to up regulate (BDNF) in Alzheimer mouse model (Hou et al., 2010) and in acute hypobaric hypoxia (HH) condition (Liu et al., 2015). Hence, increased BDNF expression following quercetin treatment might benefit in impaired calcium homeostasis.

Docosahexenoic acid is the omega-3 polyunsaturated fatty acid (PUFA), found in the cell membranes. DHA has been found to have pleiotropic effects and it is owing to the multiple target sites. It has been known to have anti-inflammatory (Belluzzi et al., 2000), anti-apoptotic, neuroprotective (Lim et al., 2005), anti-oxidant effects in different conditions in vitro and in vivo (Bourre, 2004). Cardiolipin, a mitochondrial inner membrane component interacts with cytochrome C, a mobile carrier located between mitochondrial respiratory complex III and complex IV. Cardiolipin depletion detaches cytochrome C from the inner membrane. DHA accumulates in Cardiolipin and its amount influences the electron transport chain efficiency and thus ATP production (Watkins et al., 1998). DHA regulates intracellular calcium as well (Sergeeva et al., 2005; Begum et al., 2012)

In spite of the extensive data available on the Quercetin and DHA suggesting their beneficiary effects in inflammation and mitochondrial functions in other cell types, their dose dependent effects in astrocytes is still largely not clear. Thus the present study highlights the effects of Quercetin and DHA in mitochondrial dysfunction mediated by MeCP2 deficiency and in LPS activated Astrocytes.

Major Objectives:

1. To determine the effects of Quercetin on mitochondrial dysfunction mediated by MeCP2 deficiency in Astrocytes.
2. To determine the effects of DHA on mitochondrial dysfunction mediated by MeCP2 deficiency in Astrocytes.
3. To evaluate anti-inflammatory role of Quercetin on LPS activated Astrocytes.
4. To evaluate anti-inflammatory role of DHA on LPS activated Astrocytes.

Results and Discussion:

1. To determine the effects of Quercetin on mitochondrial dysfunction mediated by MeCP2 deficiency in Astrocytes.

To investigate whether Quercetin can modulate gene and/or protein expression of mitochondrial respiratory complexes in impaired mitochondria, systematic study was carried out. Rat brain cortical astrocytes were isolated following established protocol (Chen et al., 2007) and rat C6 glioma cell line (a model for

Astroglia) was procured from NCCS, Pune. Cells were immuno characterized by glial fibrillary acidic protein (GFAP), an astrocyte marker and calcium-binding adaptor molecule 1(Iba-1), a microglial marker immunostaining and purity of astrocytes was observed to be 98-100%. In Rett syndrome mouse MeCP2$^{-/y}$model and patients, increased transcript levels of electron transport chain complexes, enzyme activities and decreased ATP levels are documented (Saywell et al.,2005;Kriaucionis et al.,2006; Pecorelli et al.,2013). MeCP2 knock down using MeCP2 siRNA was established in the present study. MeCP2 knock down & mitochondrial complexes impairment were confirmed by semi quantifying transcript levels of MeCP2, Uqcrc-1 (complex-3), Ndufv-2 (complex-1) and GAPDH (internal standard) by RT-PCR, protein levels by western blot and respiratory complex-I: NADH: ubiquinone reductase and complex-III: Coenzyme Q – cytochrome c reductase enzyme activities assays. Uqcrc-1 and Ndufv-2 genes expression were up regulated significantly in MeCP2 knock down Astrocytes compared to negative control. Ndufv-2 protein level was also increased in MeCP2 deficient Astrocytes. Mitochondrial electron transport chain complex- I activity was higher whereas complex-III activity was lower compared to control.

To assess the role of Quercetin(QH) in MeCP2 knock down Astrocytes, (25 & 100 μM) concentrations were selected. After 12h pre-incubation of quercetin, MeCP2 siRNA for 24h treatment using hiperfect reagent was carried out as per the manufacturer protocol. The gene expression study showed normalized Uqcrc1 and Ndufv2 levels in QH treated MeCP2 knockdown while compared to MeCP2 knockdown and control groups. BDNF gene expression was observed to be reduced in MeCP2 knockdown but it was equivalent to control in QH treated MeCP2 knockdown astrocytes. This indicates that Quercetin can regulate BDNF expression by sensing the deficiency. GFAP gene expression was also down regulated in QH treated MeCP2 knockdown Astrocytes than MeCP2 knockdown alone. Respiratory electron transport chain complexes protein expressions study by western blot and enzyme activities by spectrophotometric methods is being carried out in present.

Mitochondrion is one of the calcium stores and study of intracellular calcium is an indirect evidence of its functioning. Intracellular calcium was also quantified in (25, 50,100 & 200μM) QH treated MeCP2 knockdown C6 glioma cells using Cal520AM calcium dye. MeCP2 knock down cells showed significantly high cytosolic calcium while compared to negative control whereas 25, 50,100 μM Quercetin treated MeCP2 knockdown C6 cells showed respectively $p<0.\ 0.1$, $p<0.1$ and $p<0.001$ lowered $[Ca]_{cyt}$ in comparison to untreated MeCP2 knock down cells. 200 μM Quercetin increases $[Ca]_{cyt}$ in Normal control and MeCP2 knock down cells but interestingly the$[Ca]_{cyt}$ difference in 200μM QH treated MeCP2 knock down cells to normal control is lower than difference between normal control and 200μM QH treated control cells. This suggests that

Quercetin at higher doses also acts in cytosolic calcium concentration dependent manner. To ascertain the underlying mechanism of QH's effect on $[Ca]_{cyt}$ in MeCP2 knock down Astrocytes, BDNF transcript level was evaluated as it's a regulator of Calcium channel and one of the target genes for MeCP2. Uqcrc1 and Ndufv2 transcript levels were also assessed in calcium chelated groups to check the interconnected effect of calcium flux on mitochondrial respiratory complexes genes expression.

Previous studies have shown increased ROS generation in MeCP2 mutated Rett syndrome model (Felice et al., 2014). Since QH is a known antioxidant, (QH 25, 50,100 & 200 μM) dose dependent effects in MeCP2 knock down C6 glioma cells were investigated by DCF-DA staining. Fluorimetric intensity analyses indicated increased ROS generation in MeCP2 knock down C6 cells. QH100 & 200 μM treated MeCP2 knock down C6 cells significantly reduced ROS production compared to untreated MeCP2 knock down C6 cells.

Cytosolic calcium was shown to be increased along with apoptosis. To evaluate if the increased cytosolic calcium level points to apoptosis, MTT cell viability assay was performed. Data showed no cell death in any of the groups.

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

In this study, dose dependent effect of Docosahexaenoic acid (DHA) on mitochondrial electron transport chain complexes genes, proteins and enzyme activities by RT-PCR, western blot and spectrophotometry respectively in MeCP2 knockdown Astrocytes was determined. 25 & 100 μM DHA concentrations were selected following the previous published reports (Yang et al., 2013). After DHA 12h pre-treatment, MeCP2 20nM siRNA 24h incubation was carried out. The gene expression study showed down regulated Uqcrc1 levels in 25 & 100 μM DHA treated groups, whereas, it showed up regulated Ndufv2 in 100 μM DHA treated and normalized Ndufv2 in 25 μM DHA treated while compared to MeCP2 knockdown and control groups. BDNF and GFAP gene expression were observed to be equivalent to control in DHA treated MeCP2 knockdown astrocytes. These data indicate dose dependent beneficial effect of DHA on genes expression of mitochondrial electron transport chain. Respiratory electron transport chain complexes protein expressions study by western blot and enzyme activities by spectrophotometric methods is also being carried out in present as mentioned above.

Intracellular calcium was also quantified in 25, 50,100 &200 μM DHA treated MeCP2 knockdown C6 glioma cells as discussed previously. This data shows robustly increased cytosolic [Ca] in 25μM DHAtreated MeCP2 knock down

cells and decreased cytosolic [Ca] in 200μM DHA treated MeCP2 knockdown cells.

ROS generation in DHA treated MeCP2 knock down C6 glioma cells show highly increased ROS in 25μM DHA treated MeCP2 knockdown cells and decreased ROS in 200 μM DHA treated MeCP2 knockdown cells. Thus the dose dependent effect of DHA on cytosolic calcium and ROS generation is similar. In MTT assay, there was no cell death observed.

3. **To evaluate anti-inflammatory role of Quercetin on LPS activated Astrocytes.**

Quercetin has been already known to down regulate expression of certain pro-inflammatory cytokines in microglial cell line at 12.5-20μM (Sun et al., 2015) but does not affect astrocytes at those lower concentrations. Also, 25 & 50 μM Quercetin treated astrocytes showed minor difference in cytokines release compared to inflammation induced Astrocytes (Sharma et al., 2007). Hence in the present study, effects of higher QH concentrations in LPS treated rat cortical astrocytes were determined. Firstly, time dependent (for 30min, 4 h, 8 h, 12 h and 24 h) gene expression pattern of pro-inflammatory cytokines IL-1β, IL-6, TNF-α and other markers Cycloxygenase-2 (COX-2), hemeoxygenase-1 (HO-1) and Toll like receptor 4 (TLR4) in 1μg/ml LPS treated astrocytes was monitored. 24 h LPS exposure exhibited substantial increase in inflammatory markers in other cell types (Ohgami et al., 2003) but the diminished IL-1β, IL-6, TNF-α and COX-2 transcript levels were observed in present study. LPS, an endotoxin acts through TLR-4 mainly in astrocytes. TLR-4 transcript level showed insignificant change in LPS treated groups in comparison to control. IL-1β, IL-6, TNF-α, and COX-2 transcript levels illustrate bell shaped pattern, induced expression from 30min to 12 h. The highest expression of cytokines and COX-2 were at 8 h time point, henceforth it was considered to examine effects of Quercetin doses.

Pro-inflammatory cytokines IL-1β, IL-6 and TNF-α were ascertained after Quercetin hydrate incubation for 2 h followed by 8 h of LPS exposure. Cytokines transcript levels were unaltered at 200 μM or 25 μM Quercetin treatments while the levels reduced significantly in 100 or 50 μM doses when compared with LPS alone. COX-2 transcript level was also found to be lowered at 200, 100 and 50 μM doses and HO-1 level increased at 200, 100 and 25 μM doses. TLR-4 transcript level was not increased by 1μg/ml LPS but it was still checked in quercetin treated groups to determine if quercetin exert any effect on TLR-4 gene expression.TLR-4 transcript level was not changed in LPS or quercetin treated groups was observed.

Involvement of multiple signalling molecules operated during inflammation makes it a complex mechanism. Inflammation in Astrocytes is reported to be via MAPK, NF-κB, JAK/STAT and AP-1 pathways (Gorina et al., 2011). Bacterial lipopolysaccharide (LPS) binding to TLR 4 activates MyD88 adaptor which in turn causes early activation of NF-κB and MyD88 independent activation of MAPK. p38 regulates phosphorylation of Stat1 and the transcriptional activity of NF-κB and thereby influences LPS induced gene expression in Astrocytes (Gorina et al., 2011). The pro-inflammatory markers under p38 regulation are IL-1β, IL-6, TNF-α, MCP-1, COX-2, iNOS (Lu et al., 2010; Font-Nieves et al., 2012). In our study, phospho p38 level was found to be lowered at 200, 100 and 50 μM doses whereas up at 25 μM dose while compared to LPS alone. The transcript levels of IL-1β, IL-6, TNF-α, COX-2 were down regulated at either 200,100 or 50 μM QH but none of them were affected by 25μM quercetin which is concomitant with phospho p38 expression. Quercetin causes increased phosphorylation of p38 MAPK which also controls Nrf2 pathway at lower micromolar doses in LPS induced microglial BV-2 cell line (Sun GY et al., 2015). In this study, higher phosphorylated p38 expression and also up regulated Hemeoxygenase1 (Nrf2 pathway product) at 25μM quercetin dose was found. However, significantly increased HO-1 transcripts in higher doses suggest more up regulation of Nrf2 pathway or involvement of other pathways also in regulation of HO-1 in astrocytes via Quercetin. LPS has been documented to cause delayed ERK1/2 activation after 2h (Gorina et al., 2011). We also observed no activation of ERK1/2 after 1 h LPS incubation but phosphorylated ERK1/2 level was higher in 50,25μM quercetin treated astrocytes in 1 h in comparison to LPS alone. ERK regulates IL-1β, IL-6, TNF-α, MCP-1, iNOS transcription but up regulation of ERK1/2 in quercetin treated astrocytes indicated lowering effect only in IL-6 expression at 50μM QH whereas no effect on cytokines gene expressions at 25μM QH. Reduced pIKBα protein level in 200, 100 and 50 μM Quercetin groups whereas increased level in 25μM Quercetin group were seen. Thus, data indicates modulatory effects of quercetin on p38, ERK and NFκB signaling pathways in dose dependent manner.

4. To evaluate anti-inflammatory role of DHA on LPS activated Astrocytes.

To examine the effects of Docosahexaenoic acid, an Omega-3 PUFA in inflammatory astrocytes, cells were seeded at a density of $5*10^5$/ml and pre-incubated for 12 h with DHA (100, 25 μM) conjugated to Bovine serum albumin (BSA- 1mg/ml) followed by 6 hLPS/IFN-ϒ (LPS-5μg/ml; IFN-ϒ-100U/ml) treatment. Total RNA was collected and transcript levels of pro-inflammatory markers were measured as discussed in Objective 3. 100μM DHA treated LPS/IFN-ϒ activated astrocytes exhibited significantly higher IL-1β, COX-2 genes expression whereas IL-6, TNF-α, HO-1 and TLR-4 genes expression remained unchanged compared to LPS/IFN-ϒ alone treatment.

However, 25μM DHA treated LPS/IFN-ϒ activated astrocytes showed down regulated IL-1β and TLR-4 genes expression and a subtle difference in IL-6, TNF-α, COX-2, HO-1 compared to LPS/IFN-ϒ alone. Other studies on DHA has documented anti-inflammatory role at 100μM, 50 μM doses in other cell types which is owing to its direct or indirect effects on phosphorylation of signaling molecules (Zhao et al., 2005; Yang et al., 2013). In present study, the modulatory effects of DHA on signaling pathways MAPK and NF-kB were also ascertained by collecting total protein at 30min and 6 h LPS/IFN-ϒ incubations. The phosphorylated ERK1/2 protein expression was found to be increased in both (100, 25 μM) DHA and LPS/ IFN-ϒ 30min treated groups whereas it decreased at 6 hr time point in comparison to LPS/ IFN-ϒ group. The proteins expression of pIKBα, Phospho p38, GFAP, Caspase 3 was also performed by western blot analysis. These data showed no significant alteration in expressions that is concomitant with the genes expression studies data which showed anti-inflammatory effects only when compared to LPS/ IFN-ϒ. The recent genome-wide studies has also shown that MeCP2 could bind to methylated and unmethylated CpG DNA and facilitate activation or repression of transcription depending upon the conditions in which genes are expressed (Ma and Li et al., 2015; Baubek et al., 2013; Miao et al., 2012). LPS induces MeCP2 expression in THP-1 cells and causes inflammation by increasing cytokines release (Ma and Li et al., 2015). Thus, lowered MeCP2 expression could be partly associated with anti-inflammatory role of a compound. To evaluate the anti-inflammatory role of DHA in astrocytes, the MeCP2 gene expression was assessed by RT-PCR. MeCP2 transcript level was significantly increased in LPS/ IFN-ϒ supplemented astrocytes in comparison to control. Interestingly, 100 μM and 25 μM DHA showed decreased level of MeCP2, but negligible anti-inflammatory effect was observed through regulation of pro-inflammatory cytokine and enzymes genes expression which is a bit contradicting to the previous reports. BDNF (a growth factor), Uqcrc1, Ndufv2 (mitochondrial respiratory chain complexes genes) and GFAP (astrogliosis marker) are regulated by MeCP2 and these genes also showed altered levels. DHA (100, 25 μM) did not display highly significant anti-inflammatory effect through suppressing pro-inflammatory cytokines and enzymes expression in rat cortical astrocytes.

Conclusions:

In summary, data from current study show anti-oxidant and calcium level modulatory effects of Quercetin and DHA in MeCP2 deficient Astrocytes in dose dependent manner. Quercetin also exhibits significant anti-inflammatory effects in astrocytes by modulating MAPK and NF-κB pathways.

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Animal ethical statement:

All the mentioned studies were approved by institutional animal ethics committee (IAEC), Biochemistry Department, Faculty of Science, The

Maharaja Sayajirao University of Baroda. The protocols numbers are as follows: BC/17/2012, ZD/18/2013, ZD/02/2013, ZD/28/2014, ZD/01/2016.

Publication:

- Perspective on '**Optogenetics**' for the book DNA technology (2nd edition) published in December 2016 (Publishing group: ABC-CLIO, USA)

- **Swertisin an Anti-Diabetic Compound Facilitate Islet Neogenesis from Pancreatic Stem/Progenitor Cells via p38 MAP Kinase SMAD Pathway: an in Vitro and in Vivo Study**. **PLoS ONE** 10(6): e0128244. doi:10.1371 /journal.pone.0128244. Dadheech N, Srivastava A, Paranjape N, Gupta S, **Dave A**, Shah GM, et al. (2015).

Manuscripts: (Under preparation)

- Quercetin exerts anti-inflammatory effects through differently modulating neuroinflammatory pathways in rat cortical Astrocytes.
- Effect of Quercetin and Docosahexaenoic acid (DHA) on intracellular calcium levels in MeCP2 knock down C6 glial cells.
- Molecular mechanisms underlying modulatory effects of DHA in LPS/IFN-ϒ activated Astrocytes.
- Mitochondrial dysfunction in MeCP2 knock down cortical Astrocytes.

Presentations and conferences attended:

- Participated in **Science conclave 2017** held on 28th February 2017 at the Faculty of science, The Maharaja Sayajirao University of Baroda, Vadodara, India.
- Oral presentation entitled **'Role of MeCP2 in mitochondrial impairments and myelin defects'** in nanosymposium **'Rett syndrome'** at the 45th annual meeting of Society for Neuroscience (SfN) ' Neuroscience 2015' held from 17th to 21st October 2015 in Chicago, Illinois, USA.
- Oral presentation in IBRO/APRC sponsored **International school** on **'Mitochondria and Neurodegeneration'** held from 26th to 31st October 2015 in Panjab University, Chandigarh, India.
- **'Neuroscience research from Mechanisms to Applications'** a 33rd Annual conference of India Academy of Neurosciences (IAN) from October 31 to November 2, 2015 held in Panjab University, Chandigarh, India.
- **'Molecular basis of Diseases'** seminar during 1st& 2nd December 2014 organised by Department of Biochemistry, The M. S. University of Baroda, Baroda, India.
- Poster '**Role of Methy-CpG-binding protein 2 (MeCP2) in impaired mitochondrial functions in glial cells**' in the National conference

'**Neurodegenerative disorders & Metabolic diseases'** organized by Society for Neurochemistry-India (SNCI-2014) from 10th to 12th September 2014 .

- Poster presentation entitled '**Molecular mechanisms underlying modulatory effects of Quercetin in LPS activated rat cortical astrocytes'** at FENS featured regional meeting from September 11-14, 2013 held in Prague, Czech Republic.
- Poster presented entitled **'Effects of Quercetin on LPS activated rat cortical astrocytes'** at National conference on **Endocrinology and Reproduction: Innovatives in Reproductive Biotechnology**, a 31st meeting of the SRBCE at Karnataka University, Dharwad, February 2013.

Training schools attended:

- IBRO-APRC advanced school on **'In vitro and in vivo recording of synaptic plasticity in health and diseases'** held from April 29th to May 11th, 2017 at Tarbiat Modares University and Shahid Beheshti University, Tehran, Iran.
- IBRO-APRC associate school on **'Mitochondria and Neurodegeneration'** held from 26th to 31st October 2015 at Punjab University, Chandigarh, India.
- Pre-conference workshop on**'Trends in Neurorepair and Biobehavioural Research'**held from 3rd-9thSeptember 2014 at CEFT, Sri Ramachandra University, Chennai, India.
- Pre-congress training school on **'Stem cells and biomaterials in regenerative medicine'**held from 9th to11th September 2013 at the Institute of Experimental Medicine ASCR, Prague, Czech Republic.

Honours and achievements:

Sr. No.	Event	Award	Year
1.	IBRO-APRC advanced school on 'In vitro and in vivo recording of synaptic plasticity in health and diseases' in Tehran, Iran	IBRO/APRC Travel grant	2017
2.	45th annual meeting of Society for Neuroscience (SfN) 'Neuroscience 2015' in Chicago, Illinois, USA.	SERB international Travel grant (SERB's Commitment Letter No. & date: ITS/3877/2015-16, Dated: 17-09-2015)	2015
3.	IBRO/APRC sponsored International school on	IBRO/APRC Grant	2015

	'Mitochondria and Neurodegeneration' in Punjab University, Chandigarh, India		
4.	28th Annual meeting of Society of Neurochemistry (SNCI, India) held at Chennai, India	Best poster award (First author)	2014
5.	Pre-congress training school on 'Stem cells and biomaterials in regenerative medicine' held at Prague, Czech Republic	IBRO-WERC grant	2013
6.	FENS featured regional meeting at Prague, Czech Republic	DBT travel grant	2013
7.	National Conference on Endocrinology and Reproduction: Innovatives in Reproductive Biotechnology' Dharwad, India.	3rd place for poster	2013
8.	**GATE** (Graduate Aptitude Test in Engineering) score: 97.03 Percentile (All India rank: 375, Jan 2013), 92.42 Percentile (All India rank: 790, Jan 2010)		

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