CHAPTER 3

SCOPE AND PLAN OF THE STUDY

3.1. SCOPE

Developing therapeutic treatments for ischemic stroke is an intrinsically difficult endeavor because of the heterogeneity of the causes, the anatomical complexity of the brain, and the practicalities of the victim receiving both timely and effective treatment. This should in no way be a disincentive to research, but instead, a clarion call to intensify efforts to ameliorate suffering and death from this common health catastrophe. There is much evidence pertaining to the wide beneficial health effects of flavonoids and, in particular, their ability to protect the brain from ischemia-induced damage. Flavonoid compounds are receiving significant attention as therapeutic multifunctional cytoprotective agents with diverse biological effects acting at multiple sites by prophylactic action on prevention of stroke rather than as an acute therapy. The scope of this study is to assess and put into perspective salient features of the beneficial action of total oligomeric flavonoids in \textit{in vitro} model of neuronal damage in PC12 and in rat model of transient focal ischemia and to discuss a scenario concerning their potential, in drug combination, to target distinct events like apoptosis, neurochemical and biochemical alterations following IR including the relative neurological and behavioural changes in the quest for a disease modifying therapy.
3.2. PLAN OF THE STUDY

The study was planned and executed in various phases as mentioned below.

PHASE I

- Literature review
- Selection of plant material *Cyperus rotundus* and extraction of the rhizomes with methanol.
- Preparation of Total Oligomeric Flavonoids (TOFs) from rhizomes
- Qualitative analysis of CR and TOFs
  - *Preliminary phytochemical analysis*
- Quantitative analysis of extract of CR and TOFs
  - *Phenol content*
  - *Flavonoid content*
- HPTLC fingerprinting of extract of CR and TOFs
  - *Quantification of Quercetin and Catechin*
- *In vitro* free radical scavenging of CR and TOFs
  - *In vitro DPPH radical scavenging assay*
  - *In vitro lipid peroxidation assay*
  - *In vitro nitric oxide scavenging activity*
  - *In vitro ABTS radical scavenging assay*
  - *In vitro superoxide radical scavenging activity*
PHASE II

- Effect of TOFs against NMDA induced neuronal damage
  - Optimization of experimental conditions for PC12 differentiation
    - PC12 cell culture and differentiation
    - Isolation of mRNA and RT-PCR analysis of Neurofilament
  - In vitro cytotoxicity assessment in PC12 cell lines by MTT assay
    - NMDA
    - Memantine
    - CR Extract
    - TOFs
  - Effect of memantine and TOFs on NMDA induced neuronal damage
    - Assessment of cell viability.
      - MTT assay
      - Lactate dehydrogenase release (LDH)
    - Effect of TOFs on Reactive Oxygen Species (ROS) generation using DCF-DA and fluorescence imaging
    - Expression of apoptotic markers by RT PCR in PC12 cell lines
      - BAX
      - BCl-2
      - Caspase 3
PHASE III

- Effect of TOFs on MCAO induced transient focal ischemia in rats
  - Neurological deficits
  - Sensorimotor functions
    - *Initiation of walking*
    - *Turning in an alley*
    - *Beam balance*
    - *Visual placement*
  - Behavioural functions
    - *Elevated plus maze test*
    - *Open field exploratory test*
  - Neurochemical analysis
    - *Glutamate content*
  - Biochemical analysis
    - *Brain glutamine synthetase activity*
    - *Brain Na+K+ATPase activity*
    - *Brain superoxide dismutase activity*
    - *Brain reduced glutathione content*
    - *Brain MDA content*
    - *Protein*
  - Histopathology
3.2.1. Schematic overview of plan of the study