RESEARCH ENVISAGED
 Since late 1990’s focus of drug industry is skewed towards development and evaluation of natural molecules for ailments like cancer, neurodegeneration and other disease pathologies involving chronic inflammation. Nowadays, 80% of the world’s population uses medicines which are directly or indirectly derived from plants and they make up a 25% share of the pharmaceutical arsenal. The fleet is led by the flagship drug Taxol, which has been approved by the FDA for the treatment of several human malignancies (Walsh and Goodman, 2002). Many other drugs originally discovered from nature have also been approved by the FDA, including camptothecin, vinblastine and vincristine, doxorubicin and the bleomycins (Wani et al., 1987; Wani et al., 1986). Several other compounds of natural origin such as resveratrol (Azmi et al., 2005; Goswami and Das, 2009), curcumin (Goel et al., 2007; Surh and Chun, 2007), thymoquinone (Banerjee et al., 2010) and epigallocatechin have shown potential in treating various diseases (Ahmad et al., 1997; Hussain et al., 2005; Katiyar et al., 1992; Nihal et al., 2009).

Despite promising results in preclinical settings, the applicability of these agents in humans has met with only limited success, largely due to insufficient systemic bioavailability (BA) (Anand et al., 2007; Boocock et al., 2007). Latter is majorly due to compromised solubility, poor stability in solution and/or physiological settings, limited absorption, high metabolic conversion to inactive metabolites, fast elimination, preferential distribution into tissues or a combination of these factors. The modern delivery technologies like nano-based encapsulation technique can however perk up the BA and performance of these molecules of natural origin.

It will be worthwhile to suitably optimise the value of existing tradition based knowledge bank of folklore medicines rather than trying to synthesize new drug entities. Making renewed presentation of these molecules, by giving them a pharmaceutical couture, represents a strategy proposed by us in this research work.

Among the nanoparticulate systems, the advantages speculated with the solid lipid particle (SLN) technology motivated us to explore its potential for achieving high systemic concentration of the entrapped drug(s) and deliver it across the blood brain.
Research Envisaged

barrier (BBB) (Kaur et al., 2008). SLNs are colloidal drug nanocarriers consisting of spherical solid lipid particles in the nanometer range, dispersed in water or in an aqueous surfactant solution. SLNs have been reported as suitable systems for enhancing the BA of quercitin (Li et al., 2009a) and vinpocetine (Luo et al., 2006).

Curcumin (log p: 2.38; mol wt: 368.38) has all the desirable features of a desk-designed, multipurpose drug with pluripharmacological properties. A poor solubility and BA and a debatable pharmacokinetic profile exempts it from being approved as a drug. Formulating curcumin for clinical efficacy presents many challenges assigned to its poor physicochemical properties. Inspite numerous formulation challenges, several strategies such as nanoparticles, liposomes, complexation with phospholipids and cyclodextrins and solid dispersions have been developed to improve the BA of curcumin (Anand et al., 2010; Bisht et al., 2007; Maiti et al., 2007; Shoba et al., 1998; Tiyaboonchai et al., 2007; Tsai et al., 2011).

Curcumin plays a major protective role against neurodegeneration (Bala et al., 2006; Cole et al., 2007; Sethi et al., 2009). There is a body of convergent evidence suggesting that oxidative damage plays an important role in the pathogenesis of neurodegeneration including ageing, Alzheimer’s disease (AD), depression and anxiety. In light of antioxidant, anti-inflammatory, and anti-amyloid effects of curcumin, it has become a candidate compound for the prevention of neurodegeneration. In the present study, we propose to develop solid lipid nanoparticles of curcumin (C-SLNs) to achieve high plasma and brain concentrations. Further to this, considering large databank on anticancer potential of curcumin, we conducted in vitro apoptotic and mechanistic studies to observe the effect of prepared lipidic nanoparticles on improvising and maintaining the anticancer potential of free curcumin.

Recent studies from our lab and from other labs have shown that sesameol a very efficient antioxidant (Thiraviam et al., 2009) possesses chemopreventive, antimitagenic, hepatoprotective and anti-ageing properties (Kapadia et al., 2002; Kaur and Saini, 2000). Sesameol is a small molecule (mol wt. 138.28) with a water solubility of 38.8 mg/ml and is rapidly metabolized and cleared from the body within 0-4 h (Jan et al., 2009). Moreover, its log P is close to the lower limit (log P sesameol =
Research Envisaged

1.29) required for good CNS permeation (recommended log P=2.0±0.7 (Earll, 1999)), and its conjugation to glucuronides and sulphates reduces its permeability across the BBB. A suitable carrier which can deliver effective amounts of sesamol to brain in an unchanged form is thus desired.

Furthermore, carcinogenic potency database indicates its potential to cause forestomach cancers in rodents with TD\textsubscript{50} values of 1.35 and 4.5 g/kg/day dose in mice and rats respectively. The effect (may be attributed to its irritant nature) is not of much concern considering that there are no forestomachs in humans and the TD\textsubscript{50} dose is much higher than the pharmacological per oral dose of 50-100 mg/kg/day (http://potency.berkeley.edu/chempages/SESAMOL.html). The observation however reinforces the need of a suitable carrier system which may carry the drug to systemic circulation and finally to brain without effective free concentration build up in the GIT. With this prelude in mind, it is proposed to develop sesamol loaded SLNs and conduct its pharmacokinetic studies to confirm its delivery to the brain.

We also wish to scale-up (≥1L) the lab scale process of preparing SLNs and match the particle size, total drug content, % entrapment efficiency, transmission electron microscopy, infrared spectroscopy, differential scanning calorimetry, powder X-ray diffraction studies and in vitro drug release of scale up (100X) batch with the 1X lab batch. This was especially conceived, considering the fact that the method of microemulsification followed by addition into cold water (0-4°C), used presently by us for preparing the SLNs, produces very dilute and small batches of SLN dispersions (5 ml microemulsion diluted to 50-100 ml). Hence production of denser dispersions (reducing the dilution factor from 1:20 to around 1:1 to 1:5) followed by their scale-up, was proposed.

OBJECTIVES OF THE STUDY AND PLAN OF WORK

We plan to prepare curcumin and sesamol loaded SLNs, ensuring a small particle size with high entrapment. Step wise details of the proposed protocol are given below:

1. **Development of Solid Lipid Nanoparticles (curcumin/sesamol)** using microemulsification technique.

2. **Characterization of the developed SLNs (curcumin/sesamol).**
Research Envisaged

- Photon Correlation Spectroscopy (PCS) for measuring particle size or distribution.
- Transmission Electron Microscopy (TEM) for surface characterization.
- Differential Scanning Calorimetry (DSC) to confirm incorporation of drug SLNs.
- Infrared Spectroscopic Analysis (IR) to confirm the formation of SLNs.
- Powder X-Ray Diffraction (PXRD) to determine crystalline/amorphous nature of developed SLNs.
- Determining the total drug content, drug entrapment efficiency and in vitro release of the developed SLN formulation(s).

3. **Scale-up of sesamol loaded SLNs to 100X batch (1 litre)**

4. **In vitro studies of curcumin and curcumin loaded SLNs on cancer cell lines**
   - MTT Assay
   - DNA Fragmentation
   - Mitochondrial Membrane Potential
   - Caspase Activity (caspases 3, 8 and 9)
   - Immunoblotting Proteins

5. **In vivo studies**
   - Pharmacokinetic studies in rat plasma using LC-MS/MS for curcumin and HPLC to quantify sesamol in plasma and brain of mice.
   - Proof of concept for delivery of SLNs to brain using Confocal/Fluorescence microscopy.
   - Biodistribution and gamma scintigraphy studies to estimate the distribution of SLNs after administration by oral and i.v route.
   - Pharmacodynamic evaluation of developed curcumin loaded SLNs in models of neurodegeneration (Alzheimer’s/Depression).