Chapter 1

Introduction

Herbal drug can be defined as any plant or its organs containing pharmacologically active substances which are used for various clinical purposes.The use of herbal extracts as a medicine is gaining attention because of their low or no side effects and mixed pharmacological actions as compared to synthetic drugs (Wagner and Ulrich, 2009). 75 – 90% of the rural population in developing nations relies on herbal medicine for their health care as they are easily available. Herbal drugs can be used in the form of extracts or phytochemicals. Extracts such as artichoke leaf extract (Wittemer et al. 2005), *Cimicifugafoetida* extract (Gai et al., 2012), *Andrographispaniculata* extract (Sermkaew et al. 2013), *Ginkgo biloba* extract (Chen et al. 2010) and phytochemicals such as curcumin (Beevers et al. 2011), berberine (Vuddanda et al. 2010), paclitaxel (Potemski and Płuzańska1999) are widely used in the treatment of several diseases.

*Ficusreligiosa*L. is one such herbal drug possessing several pharmacological actions. It is included in ayurvedic formulations such as nyagrodhadichurna, nyagrodhadikashayam and saribadyasavam along with other herbal drugs for the

treatment of diabetes, cancer, inflammatory condition, neuronal disorders, and effective against several microbes (Pandit et al. 2010). *Ficusreligiosa*L. is a sacred tree and it is native to India. It belongs to the family, Moraceae. It possesses strong anti-oxidant activity and comes under the class of rasayana (rejuvenators and stress relievers). Several phytochemicals have been reported in *Ficusreligiosa*L. include β-sitosterol, stigmasterol, lupeol, campesterol, lanosterol, vitamin K, n-octacosanol, metyloleonate, lupen-3-one, bergapten, bergaptol, amino acids, myricetin, quercetin, kaempferol, phenolic compounds undecane, tridecanemtertradecane, (Z)-3-hexenol, monoterpenes, aliphatic alcohols and triterpene alcohols(Singh et al. 2011).

In spite of the several pharmacological actions of *Ficusreligiosa*L., its applications are limited due to the difficulty in carrying out qualitative and quantitative analysis of all the phytochemicals present in it. Further, the need of reference compounds for each phytochemicals present, results in higher cost.Hence, the present research was focused on the qualitative and quantitative analysis of the marker compound present in*Ficusreligiosa*L. extract which is both cost effective and time effective. Also due tounavailability of pharmacokinetic parameters of the marker compound or other phytochemicals present in *Ficusreligiosa*L. extract,its use is limitedfor extensive research. In addition to the bioavailability issue, higher dose requirement, delayed onset of action and frequent drug administration are the most common limitations of any extract.

Solid lipid nanoparticles (SLN) are well known for improving the bioavailability of poorly soluble drugs, sustaining the drug release and increasing the permeability to cell membranes due to their lipid nature. SLN are spherical shaped particles having the particle size ranging between 10 nm and 1000 nm. The advantages of SLN include avoidance of organic solvents, thus, avoiding toxic effects, enhanced drug loading capacity of water insoluble drugs, improved stability and achievement of controlled or

sustained release (Mehnert and Mader 2001). Solid lipids used for the preparation of SLN are triglycerides (eg. tristearin), hard fatty acids (eg. stearic acid) and acyl glycerols (glycerylbehenate). Surfactants used in the SLN formulation for stabilization include phospholipids (eg. lecithin), bile acids (eg. sodium taurocholate), poloxamer, tween and span(Muller et al. 2000).

Several research works have been carried out where SLN approach was used to improve the properties of an herbal extract. SLN form of *Calendula officinalis*extract was found to have improved re-epithelialization activity than free *Calendula officinalis*extract (Arana et al. 2015). In a different study, sage and savoury extracts were loaded into SLN and showed improved stability when exposed to gastrointestinal tract conditions (Campos et al. 2015). Encapsulation of *Brassica oleracea* L. extract into SLN preserved the degradation of anthocyanins and polyphenolic compounds (Ravanfar et al. 2017). Similarly, SLN loaded with phytochemicals have been formulated by researchers to improve their pharmacokinetic properties. Paclitaxel loaded SLN have showed improved permeability to blood brain barrier as compared to plain paclitaxel (Chirio et al. 2014). Curcumin loaded SLN were found to have 9.5-fold higher bioavailability than that of plain curcumin (Baek et al. 2017). SLN approach ofboth*Ficusreligiosa*L. or its marker compoundhave not been observed in the literature search. Further, the present study was focused on the effect of *Ficusreligiosa*L. extract loaded SLN on the oxidative stress induced diabetes based on its both antioxidant and antidiabetic activities.

Diabetes is a group of metabolic disorders resulting in higher plasma glucose level. Around 9 % of the world's adult population is suffering from diabetes (Meetoo et al. 2007). Diabetes will be the 7th leading cause of death by 2030.Type 2 diabetes (T2DM)accounts for the 90% of the diabetic cases while the other 10% contributes to type 1 diabetes and gestational diabetes (WHO 2013). T2DM primarily occurs due to

insulin resistance and relative lack of insulin. The most common symptoms of T2DM include increased thirst, increased hunger, frequent urination, tiredness and weight loss. Patients suffering from T2DM have reported other vascular complications such as diabetic neuropathy, diabetic nephropathy as a result of it (Cade 2008). Among different mechanisms involved in the diabetes, oxidative stress plays a major role in the pathogenesis and progression of diabetes (Kelley et al. 2002).Mitochondria are the major sites of reactive oxygen species (ROS) generation by electron transport chain (ETC) (Ernster and Schatz 1981).

Mitochondria function by synthesizing ATP molecules (Addabbo et al. 2009). Glucose from food enters blood and undergoes glycolysis to form pyruvate; through tricarboxylic acid (TCA) cycle, pyruvate molecules are converted into oxaloacetate with the liberation of reducing equivalents. These reducing equivalents are utilized by a mitochondrial pathway, ETC, which is a main metabolic pathway in mitochondria (Krauss 2001). ETC produces ROS during conversion of reducing equivalents into ATP molecules. Normal level of ROS is necessary for cellular functions including insulin release. Further, produced ROS will be enzymatically neutralized by the antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase. When the level of ROS exceeds the levels of antioxidants, accumulation of ROS occurs (Patti and Corvera 2010).

Increased level of ROS causes mitochondrial dysfunction and insulin resistance (Paravicini and Touyz 2008). Mitochondrial overproduction of ROS has five major mechanisms in the pathogenesis of diabetes such as increased polyol pathway flux, increased level of advanced glycation end products, glucose autoxidation, increased protein kinase C activation which is inactive in normal conditions and increased hexosamine pathway flux (Araki and Nishikawa 2010, Giacco and Brownlee 2010).

Increased ROS level also increases ATP to ADP ratio in cytoplasm which results in increased intracellular Ca^{2+} ion level due to closure of ATP-sensitive K^+ channels (K_{ATP}) and decreased hyperpolarization of outward K^+ flux. This causes activation of protein kinaseswhich are responsible for cellular proliferation, differentiation, development, inflammatory responses and apoptosis and mediates exocytosis of insulin(Kashihara et al. 2010).In addition to this, increased ROS level causes 8-hydroxylation of guanine base and produces 8-hydroxydeoxyguanosine, which is a biomarker of intracellular oxidative stress *in vivo* in T2DM (Nishikawa et al. 2007). Hence, mitochondria can act as target machinery for the treatment of oxidative stress induced diabetes (Heller et al. 2012).

There are several approaches available for effective treatment of oxidative stress induced diabetes. Approaches such as conjugation of targeting moieties with anti-oxidants or anti-diabetic drugs (Dhanasekaran et al. 2005, Mustapha et al. 2010), nanoparticulate technology (liposomes, polymeric nanoparticles, gold nanoparticles, biosensor tethered nanoparticles loaded with anti-oxidants/anti-diabetic drugs) by conjugating targeting moieties with polymers used for encapsulating drugs (Prow et al. 2008, Vega et al. 2010, Samadder et al. 2013) and bifunctionalpeptides are effective in the treatment of oxidative stress induced diabetes (Szeto et al. 2008). Of the approaches, nanoparticulate drug delivery system provides the advantages of targeted delivery to the specific site, sustained release of loaded drugs, improved bioavailability of poorly soluble drugs, protection of loaded drugs from degrading enzymes, lesser side effects because of targeted delivery and thus, effective treatment (Singh and Lillard, 2009).

Mitochondrial targeting can be achieved by the use of targeting moieties such as lipophilic cations, dequalinium, rhodamine 123, Szeto-Schiller (SS) peptides and gramicidin (Trendeleva et al. 2012, Biswas et al. 2011, Wang et al. 2010, Chunta et al. 2012, Roestenberg et al. 2012). Triphenylphosphonium is a lipophilic cation which is

most commonly used in mitochondrial targeting and it was used as a mitochondrial targeting moiety in the present research.

At the time of conceptualisation of the present research, there was no reported work on mitochondrial targeting of extract (which possesses both antioxidant and antidiabetic activities) loaded SLN for the management of oxidative stress induced diabetes. Considering the problems associated with the use of *Ficus religiosa* L. extract and the need of targeted delivery to mitochondria for the treatment of oxidative stress induced diabetes, this research work was designed to develop SLN loaded with *Ficus religiosa* L. extract. Further, the effect of *Ficus religiosa* L. extract loaded SLN on oxidative stress induced diabetes was compared with its maker compound, lupeol loaded SLN to evaluate the efficiency and toxicity of both extract form and phytochemical form.