Chapter 1

Introduction and Objectives

1.1. Background

Faba bean is botanically known as *Vicia faba* L. (Hanelt and Mettin, 1989) and belongs to the family *Leguminosae* or *Fabaceae*. It is usually grown in the winter season. Faba bean (*Vicia faba* L.) is among the oldest crops and third most essential feed grain (staple pulses) in the world. Seeds of Faba bean contains 22.4–36% protein, 57.8–61% carbohydrates, 12% fiber and 1.2–4% lipids (Baginsky et al., 2013). Different mineral elements such as calcium, iron and zinc and other minerals are also present in faba bean (Sandberg, 2002). Faba beans are a rich source of phenolics antioxidants and many bioactive compounds (Troszynska and Ciska, 2002). Polyphenols in Faba beans are extensively distributed in several parts of the plant (e.g. leaves, roots, and seeds) (Baginsky et al., 2013). As the name suggests that polyphenols are also known as polyhydroxyphenols and it is characterized by the presence of large multiples of phenol structural units (Rice-Evans et al., 1997). Polyphenols are classified into 15 classes, according to the number of phenol ring(s), binding properties of the aromatic ring (Tan and Ong, 2014). Flavonoids come under polyphenols and can be further sub-divided into flavonols, flavones, flavanones, catechins, anthocyanidins and isoflavones (Bravo et al., 2006). These compounds are widespread virtually in all plant foods, often at high levels and include phenols, phenolic acids, and flavonoids (Pietta, 2000). Many researchers have reported the therapeutic potential of polyphenols from faba beans such as antioxidant, anti-inflammatory and anti-diabetic properties (Choudhary and Mishra, 2017; Choudhary and Mishra, 2018a; Choudhary and Mishra,

2018b; Mejri et al., 2018; Turco et al., 2016). Faba bean having polyphenols are suitable food for diabetics and may prevent heart disease and reduce levels of blood glucose (Rizkalla et al., 2002)**.**

1.2. Polyphenols

Polyphenols are polyhydroxyphenols and it's having antioxidant properties help to protect chronic diseases from free radical damage. Polyphenols in cereals and legumes have been receiving considerable attention largely because of their adverse influence on color, flavor, and nutritional quality. These compounds belong to the flavonoid and tannin groups and are mostly located in the seed coat or pericarp of the grains. Polyphenols (phenolic compounds, phenolic acids such as gallic acid, cinnamic acid, and flavonoids) are considered to be secondary metabolites of the shikimate pathway and phenylpropanoid metabolic pathway. Observations made by researchers that dietary phenolics offered better protection against chronic diseases such as cancers, cardiovascular diseases, cerebrovascular diseases, diabetes, aging and neurodegenerative diseases (Scalbert et al., 2005).Structure of polyphenolic compounds is responsible for physical, chemical and biological properties.

1.3. *Diabetes mellitus (DM)*

Diabetes mellitus (DM) is like syndromes and may be results of the improper balance of glucose homeostasis. It is a metabolic disorder of carbohydrate, fat and protein metabolism. Approximately 220 million people are suffering from these diseases globally. (Colberg et al., 2010) and the number increase to double between 2005 and 2030 (Mathers and Loncar, 2006). India is rich in valuable natural resources of fruits, vegetables, cereals, herbs, and medicinal plants many of which remain

unexploited and are limited for regional or rural use only. *Vicia faba* bean is one such example and nutritionally rich staple pulses of the less privileged society of India.

Diabetes mellitus (DM) are divided generally into two categories presently known, one being type 1, which is characterized by inadequate insulin production and the other being type 2, that results from the incompetence of insulin (Nathan et al., 2009). In type-2 diabetes, there is a gradual development of insulin resistance and beta cell dysfunction, associated with obesity and an imbalance lifestyle (Zimmet et al., 2001). Type-1 diabetes is an autoimmune disease due to T-cell mediated destruction of the pancreatic beta cells (Donath et al., 2003). 90% of the total cases account for type 2 *diabetes* (Zimmet et al., 2001). Type 2 diabetes is characterized by an abnormal postprandial increase of blood glucose level, known as hyperglycemia or raised blood sugar, (Dea, 2011). Diabetes mellitus may be the result of oxidative stress or excessive free radical formation.

1.4. Relation of oxidative stress with diabetes mellitus

Oxidative stress is the result of the excessive generation of free radical in the human body. Antioxidants have the ability to counteract or detoxify the harmful effects of free radicals (Urso and Clarkson, 2003). Oxidative stress may be one of the factors in the development of diabetes, cardiovascular problems, and many other diseases (Giugliano et al., 1995). A person suffering from Type 2 diabetes mellitus (DM) found to have increased oxidative stress level (Aydın et al., 2001). Hyperglycemia enhances the generation of free radicals leading to oxidative stress when excessive free radicals react with proteins and DNA (Valko et al., 2007) and increase plasma insulin and lipid concentrations along with cardiovascular disease risk factors (Björntorp, 1990). Long term hyperglycemia could develop a variety of vascular complications due to excessive production of reactive oxygen species (ROS). Inhibition of excessive free radicals by

polyphenols in diabetic patients would be one strategy in treating diabetes mellitus and also its vascular complications (Asmat et al., 2016; Bonnefont-Rousselot, 2004).

1.5. Exploring the potential of natural products

Natural products derived from plants (herbs, shrubs, and trees) play a vital role in the treatment of type 2 diabetes, particularly in developing countries where most people do not have a facility for modern treatment (Saklani and Kutty, 2008). Natural product based drugs are cost-effective and can be easily available for poor peoples in rural areas. Exploration of natural product-based enzyme inhibitors is also encouraged in developed countries because there is anxiety about the critical adverse effects of synthetic pharmaceutical agents (Gurib-Fakim, 2006). Literature also authenticates that long term treatment and a side effect of the available synthetic medications leading towards huge demand for efficacious decreased side effects and affordable agents for the treatment of a diabetic condition (Modak et al., 2007). Different types of synthetic drugs (insulin analogs, sulphonylureas, biguanides, DPP4 inhibitors, thiazolidiones inhibitors, xanthine oxidase inhibitors are commercially available in the market for the treatment of diabetes mellitus (Barf et al., 2002) but their mechanism of counteracting effect for this increased glucose level is different for different categories (Vilar et al., 2010).Therefore, it must explore naturally occurring α-amylase, α-glucosidase, xanthine oxidase inhibitors for the treatment of diabetes mellitus. Present investigations on polyphenols derived from faba bean may be effective for overcoming hyperglycemia-induced chronic diseases, such as type 2 diabetes.

1.6. General strategies for antidiabetic activity

An important therapeutic approach for treating type 2 diabetes is to decrease the post-prandial hyperglycemia by retarding the absorption of glucose through the

inhibition of the enzymes, α-amylase, and α-glucosidase, in the digestive tract. Literature suggested that enzyme inhibitors delay the rate of glucose absorption by preventing carbohydrate digestion and consequently dulling the postprandial plasma glucose rise (Ali Asgar, 2013). It has been reported that the inhibition of starch digestive enzymes by synthetic agents, such as acarbose is an important clinical strategy for controlling postprandial glycemia (Lo Piparo et al., 2008).

Antioxidant therapy can be possibly one of the strategies for controlling *diabetes mellitus*. Since the overproduction of oxidants (reactive oxygen species and reactive nitrogen species) in the human body is responsible for the pathogenesis of some diseases. Antioxidants may act at different levels, inhibiting the formation of ROS or scavenge free radicals, or increase the antioxidants defense enzyme capabilities. Researchers have been reported that the antioxidant effect of the extract is also contributed to the antidiabetic effect(Rahimi et al., 2005).

In addition to *in vitro* study, molecular docking and simulation study provide useful information about protein-ligand interaction. Predicting the target sites of molecules using system biology approaches will be valuable and time efficient in pharmaceutical applications to make elimination of undesired biomolecules, and also avoid costly late-stage preclinical and clinical failures (Neužil et al., 2012). It covers and identifies the lead candidate, binding pocket, determination of target structure, and evaluation of the potential lead candidate (Bleicher et al., 2003). Based on this information, the present study also aimed to evaluate the inhibitory action of polyphenols against some target protein like alpha-amylase, alpha-glucosidase, and xanthine oxidase.

1.7. Motivation and significance of research work

Polyphenols derived from natural resources have high nutritional values, great structural diversity and economically feasible that motivated for searching a new lead compound for drug discovery. Faba bean is underutilized legume in India and its subcontinents. To my knowledge, a very little of work has been reported on polyphenols derived from faba beans as alpha-amylase, alpha-glucosidase, xanthine oxidase inhibitors. *In vitro* interaction of alpha-glucosidase, alpha-amylase, xanthine oxidase with polyphenols from faba beans were validated through computational biology approaches in the present studies on enzyme kinetics, molecular dynamic simulation, oxidative stress and glucose uptake in yeast cells and cell lines may be useful in revealing the importance of polyphenols from faba bean as a drug candidate.

Reflection of enzyme behavior in the presence of catechin and gallic acid were evident as conformational changes happened near binding pocket with some binding residues. These findings are valuable for understanding the behavior of ligands in the presence of α-glucosidase, alpha-amylase, and xanthine oxidase. Recently, dietary polyphenols from plants have been proposed to be unique nutraceutical supplements for diabetes patients due to their various therapeutic properties (Bahadoran et al., 2013). Dietary polyphenols based research may be helpful in the development of a drug for diabetes mellitus with cost-effective, high potency, efficacy, safety, and purity. Therefore, an attempt was made to achieve the following objectives :

1.8. Objectives

1. Extraction, purification, and characterization of polyphenols from faba beans.

2. *In vitro* and *in silico* evaluation of dietary polyphenols from faba beans as an antidiabetic agent.

I. *In vitro* and *in silico* interaction of polyphenols with porcine alpha-amylase and evaluation of the antidiabetic activity.

II. *In vitro* and *in silico* studies of polyphenols from faba beans as alpha-glucosidase inhibitors.

III. *In vitro* and *in silico* interaction of polyphenols with xanthine oxidase and evaluation of antioxidant activity

3. Glucose uptake and oxidative stress studies of polyphenols from faba beans in *Saccharomyces cerevisiae* 2376.

4. Glucose uptake and oxidative stress studies of polyphenols from faba beans on 3T3-L1 cell line.