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1.0 Diabetes

Diabetes mellitus (DM) has been known since many years ago and Sushruta has mentioned the sweetness of diabetic urine in Ayurveda (http://ekikrat.in/ayurveda_diabetes). DM is a chronic metabolic disorder with multiple etiologies, shares the phenotype of hyperglycemia with disturbances of carbohydrate, fat and protein metabolism (Solomon et al., 2012). Recently, the prevalence of diabetes has increased enormously across the globe and in the present scenario; it’s a worldwide public health problem. It is considered to be one of the major leading causes of death of human (Chauhan et al., 2010). Globally, there is a shift from communicable to chronic non-communicable diseases over the past few decades (Lim et al., 2013 and Lozano et al., 2013). Furthermore, the total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030, if successful strategies are not implemented for prevention and control (Pandey et al., 2012 and Tripathi et al., 2013). According to the International Diabetes Federation, 80% of patient lives in low and middle-income countries (Unwin et al., 2011) and about 50% of diabetes patient remain undiagnosed(Harris et al., 2000 and Mohan et al., 2005). Indian scenario suggested that about 62.4 million people are diabetes in the year 2011 according to the report of Indian Council of Medical Research India Diabetes Study (ICMR-INDIAB study) (Anjana et al., 2011) and it will be increased to 101.2 million by 2030 (Unwin et al., 2011).

1.1 Forms of diabetes

There are two forms of diabetes namely, mellitus and insipidus. Diabetes mellitus is the most common form and it is colloquially known as "Sugar diabetes"
as it results in high levels of glucose both in blood and urine. Whereas, diabetes
insipidus is caused by problems related to antidiuretic hormone

1.2 Symptoms of diabetes mellitus (www.idf.org/signs-and-symptoms-
diabetes)

The most common symptoms of diabetes are listed as, (i) excessive thirst
(polydipsia); (ii) increased hunger (polyphagia); (iii) frequent urination (polyuria);
(iv) extreme tiredness; (v) sudden loss of muscle mass; (vi) losing weight; (vii) lack
of interest and concentration; (viii) a tingling sensation or numbness in the hands or
feet; (ix) blurred vision; (x) skin infections, wounds or cuts that won’t heal; (xi)
genital itchiness and (xii) bladder infections.

Further, if diabetes is not treated /diagnosed properly, the following
symptoms, which includes, stomach ache, nausea and vomiting and heavy, rapid
breathing (Kussmaul breathing), drowsiness and diabetic ketoacidosis have also
been realized and reported (http://www.merckmanuals.com/home/hormonal_and_
metabolic_disorders/diabetes_mellitus_dm/diabetes_mellitus.html).

Other symptoms include increased levels of lipids, fatty acids and cholesterol
in blood, increased production of ketone bodies by liver. In the early stages of the
disease, symptoms may be nonexistent. That’s unfortunate, because the damage to
organs occurs even in the absence of symptoms. For this reason, it’s important for
people who may be at risk for diabetes to get their blood sugar levels checked
regularly.
1.3 Complications of diabetes

The effects of diabetes mellitus include long-term damage, dysfunction and failure of various vital organs. Diabetes is strongly associated with clinical complications either at the microvascular (due to damage to small blood vessels) and macrovascular levels (due to damage to large blood vessels). The risk of microvascular damages are blindness (retinopathy), renal failure (nephropathy), neurological complications (neuropathy), diabetic foot disorders (leading to limb amputation) and premature death. Further, increased risk of macrovascular complications like ischemic heart disease, stroke and peripheral vascular disease, cerebrovascular disease and diminished quality of life resulting in organ and tissue damage in approximately one third to one half of people with diabetes (UKPDS Group, & UKPDS Group., 1991). Numerous factors, such as genetics, environment, eating habits, physiological state, hormones and stress are considered to be associated with the development of DM (Sakurai, 2002).

1.4 Association between diabetes mellitus and alterations in metabolism of trace elements

A number of studies have shown correlation between diabetes and trace elements. Chronic hyperglycemia may cause alterations in the status of trace elements in the body and thus the essential trace elements such as copper (Fields et al., 1983, Reiser et al., 1983 and Cooper, 2012), zinc (Quarterman et al., 1966, Boquist et al., 1969, Hendricks et al., 1972, Bēgin-Heick et al., 1985 and Park et al., 1986), chromium, iron, manganese (Rubenstein et al., 1965, Hassanein et al., 1966, Everson et al., 1968 and Baly et al., 1984) and magnesium (Paolisso et al.,
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1989) are deficient in DM. Therefore, trace elements may play an important function for glucose and lipid metabolisms, particularly insulin function in DM (Hiromura et al., 2008). Excessive amounts of these metals lead to toxic effects, especially in certain genetic disorders (Zheng et al., 2008).

1.4.1 Copper and diabetes

Copper is an essential, but highly-toxic transition metal that is closely associated with regulation of biological systems in the body. It is capable of fluctuating between the oxidized Cu\(^{2+}\) and the reduced Cu\(^{+}\) state, being a co-factor for many enzymes. This divalent cation is involved in the activity of anti-oxidant enzymes (SOD) and ceruloplasmin (Vlad et al., 1993). In human, alteration in the copper homeostasis leads to tissue copper deficiency or overload states. Regulation of copper was primarily carried out by small intestine. Zinc competes with copper for intestinal absorption, so increased intake of zinc leads to copper deficiency (Song et al., 2000). Both increased and decreased Cu levels were found in diabetic patients (Isbir et al., 1993 and Trumbo et al., 2001).

1.4.2 Zinc and diabetes

Zinc is also an another essential transition metal and is found to be a cofactor of more than 200 metallo enzymes. Zinc plays a major role in the regulation of carbohydrates, lipids and proteins metabolism. Zinc is tightly bound to proteins, where, it plays a structural or catalytic role. Insulin itself is stored in an inactive form in the presence of zinc (Singh et al., 1998). Zinc ions in the secretary granules of \(\beta\) cells are known to glue insulin molecules, creating somatically stable hexamers. When the secretary granules open to the surface, the zinc ions pressure decreases
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rapidly and the pH levels change from acid to physiological level, which results in free insulin monomers and zinc ions will be released from the pancreas (Olaniyan et al., 2012). Zinc is also an integral component of Cu-Zn SOD, which acts as an antioxidant and protects from free radicals. Zinc is mainly required for normal immune function of the body.

1.5 Treatment for diabetes

As described previously, number of people suffering from diabetes has been rising steadily over the past two decades. This high prevalence deserves special attention towards the treatment of diabetes. Diabetes treatment and management strategy will help to prevent and control hyperglycemia, which includes, oral medication; insulin; transplants (Artificial Pancreas); herbal remedies; alternative treatment and change in lifestyle (Dietary modification focused on caloric reduction, exercise/physical activity and behavior therapy), etc.

Currently available therapies for diabetes include insulin and various oral antidiabetic agents such as sulfonylureas, biguanides, α-glucosidase inhibitors and glinides either used as mono or polytherapy with lifestyle alterations to achieve better glycemic control. Most of the oral diabetes medicines can have adverse effects and it varies from one class to another class of antidiabetic drugs and also variation in the patient depends on severity of disease. Moreover, injecting insulin several times in a day is painful and elevates the level of patient stress, especially in young people. Sometimes the treatment is costly and is not affordable by all class of people.
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This alarming situation became a challenge and a decision was made to research into the prevention of diabetes complications and also to develop a drug delivery system, which empowers the diabetes and non-diabetes people on how to live a diabetes-free life or cope with the diabetes if already affected. There is an urgent need to reduce the impact of diabetes on the lifespan and to improve the quality of life of people around the world. Apart from knowledge, education and awareness program, treatment is one of the key components in ensuring better prevention and control over diabetes. One of the study reports has shown, females had low awareness rates compared with males in all regions (Deepa et al., 2014). The few orthodox management options available are expensive and often associated with negative side effects; therefore, the use of indigenous medicinal plants provides better alternative, which are usually less toxic and affordable with novel technology. In recent years, a strong medical consensus has emerged in all parts of the world, which makes WHO to recommend natural herbal remedies for people with diabetes. Based on the safety and multiple targeting actions, herbal therapies are potent therapeutic means of management of DM.

1.6 Herbal remedies

Presently, there is a growing interest in herbal remedies due to the side effects associated with the oral hypoglycemic agents (therapeutic agent) for the treatment of diabetes mellitus. So the traditional herbal medicines are mainly used, which are obtained from plants, plays an important role in the management of diabetes mellitus. Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. Researchers are exploring the medicinal plants for various biological activities for
enhancing health standards of human beings and to explore herbal remedies that have traditionally been used in India to control this condition. Herbal products or plant products are rich in phenolic compounds, flavonoids, terpenoids, coumarins and other constituents, which show reduction in blood glucose levels (He et al., 2005 and Jung et al., 2006). All these herbal remedies are easily available, cheaper and they can provide a simpler, and a more natural way of controlling diabetes without any unpleasant side effects.

1.6.1 Medicinal plants used as anti-diabetic agents

Since ancient days, numbers of herbal medicines are used in the treatment and management of diabetes mellitus. Numbers of research activities have been carried out for assessing anti-diabetic efficacy of the herbal medicines. Herbal medicines for diabetes can be classified into four categories according to their mode of action, viz., (i) drug acting like insulin; (ii) drug acting on insulin secreting beta cells; (iii) drug acting by modifying glucose utilization; and (iv) drug acting by miscellaneous mechanisms.

Numbers of the most common herbs and spices are claimed to have blood sugar lowering properties that make them useful for people with or at high risk of type 2 diabetes. The following are the representative herbs, viz., *Allium sativum; Bauhinia forficata; Myrcia uniflora; Coccinia indica; Ficus carica; Ginseng; Gymnema sylvestre; Momordica charantia; Ocimum sanctum; Opuntia streptacantha; Silybum marianum; and Trigonella foenum-graecum.*

It has been understood that all the said herbs displayed a presence of a unique active constituent namely quinones at different percentage levels. The
antidiabetic efficacy of these plants may be reasoned to the quinones and other phenolics.

Thus, in order to have naturally occurring quinones, exploring the unexplored herbal medicines is necessary. Nearly more than 1500 naturally occurring quinones are available and the quinones are majorly divided into six groups and further subdivided into smaller groups based on the type and structure. Extraction of quinones from plant products is easy and moreover it is chemically stable also (Rauwald, 1990). Among all quinones, the benzoquinone, commonly known as para-quinone (oxidized derivative of 1,4-hydroquinone) have gained importance in therapeutic applications because of its bright colors (Soliev et al., 2011). The biological effect of quinones is related to its participation in redoxycles in free radical form (Hidvégi et al., 1999). Hence, the present study has been approached with the herbal plant Emelia ribes.

1.7 Embelia ribes– a source of natural benzoquinone

Medicinal plant Embelia ribes Burm commonly known as ‘Vidanga’ or ‘Vavding’ in Ayurveda, is a woody climber (Figure 1) belongs to the family Myrsinaceae. The plant is reported to possess various pharmacological activities. The main part of the plant used for therapeutic purpose is fruit, a dull red globose berry approximately 2.4 to 4.0 mm in diameter that gives brown-black color after drying. The fruit contains a thin pericarp enclosing a single seed, which is horny and reddish with a mildew-like external appearance. The term ‘fruit’ and ‘seed’ are used interchangeably with regard to its medicinal properties.
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Figure 1 Image of *Embelia ribes* plant and berries

1.7.1 Active constituents of *Embelia ribes*

Embelin (2, 5 dihydroxy-3-undecyl-1, 4 benzoquinone), an orange pigment extracted from a plant was found to be an active constituent of *Embelia ribes*. In addition to a quinone derivative, presence of an alkaloid christembine, a volatile oil and vilangin (Rao and Venkateswarlu, 1961) were in reports. Presence of other phyto constituents include fatty ingredients (linoleic acid and palmitic acid), gallic acid, simple carbohydrates (glucose and fructose), quercitol, a resinoid, tannins and glycerol (Ibrahim Khan et al., 2010 and Lakshmanan, 1990) were in reports. The structure of embelin was depicted in Figure 2.

Figure 2 Structure of Embelin (2,5-dihydroxy-3-undecyl-2,5-cyclohexadiene-1,4-dione/2-dihydroxy-3-undecyl-1, 4 benzoquinone (C₁₇H₂₆O₄)
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1.7.2 Vilangin

A dimeric form of embelin called as vilangin and it has been reported as a minor constituent of Embelia ribes berries (Rao and Venkateswarlu, 1961). It has been synthesized by reaction of embelin with formaldehyde. It occurs as a bright – orange yellow crystal, insoluble in water and soluble in organic solvents. Vilangin has been used as analytical tool in the quantitative analysis of embelin. Kiprono et al. (2004) reported, methyl vilangin from Embelia schimperi also shown larvicidal activity (yellow fever vector). List of marketed products having Embelia ribes, as one of the major components shown in Figure 3.

![Image of marketed products](image.png)

Figure 3 List of marketed products having *Embelia ribes* as one of the key components
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Embelin and its metal complexes

Rao and Venkateswarlu (1961) reported that embelin forms complexes with nearly all transition metals under suitable pH conditions. In all the cases, simple combinations of metal ions with the hydroxyls of embelin have taken place, leading to chelate structures having a fairly fixed composition in a large number of cases. Single atom complex formation was with copper, cadmium, barium, strontium, magnesium, nickel and zinc, whereas, with silver, the complex was resulted with two atoms of silver and one molecule of embelin (Dhar and Onkar Singh, 1986 & Cherutoi et al., 2005).

1.7.3 Pharmacological activities of embelin

Embelin accounts nearly 2.3 to 3.1% in *Embelia ribes* berries (Mhaskar et al., 2011). A well-known pharmacological activities of embelin includes anti-tumor, anti-inflammatory, analgesic activity (Chitra et al., 1994), anticancer (Xu et al., 2005), Chemo preventive (Sreepriya and Bali, 2005), antibacterial (Chitra et al., 2003) and antioxidant activity (Joshi et al., 2007). Bhandari and co-workers (2007 and 2009) have reported the antidiabetic, antidyslipidemic and antioxidant activity of *Embelia ribes* Burm in streptozotocin-induced diabetes in rats. Antimicrobial (Radhakrishnan et al., 2011) and UVB inhibitory activity of embelin (Radhakrishnan et al., 2012) were recently reported. Furthermore, biological properties of zinc and copper (II) embelin complexes have been evaluated and reported (Radhakrishnan et al., 2012 and 2013).

One of the etiologic factors implicated in the development of diabetes and its complications is the damage induced by free radicals and hence an antidiabetic
compound with antioxidant properties would be more beneficial. Free radicals are capable of damaging cellular molecules, DNA, proteins and lipids leading to altered cellular functions. Many experimental studies revealed that antioxidants capable of neutralizing the free radicals are effective in preventing experimentally induced diabetes in animal models. Therefore, medicinal plants with antioxidant effects may reduce the severity of diabetes and its complications.

1.8 Mode of delivery of natural benzoquinone embelin

Every drug/disease combination requires a unique challenge in delivery. Therapeutic molecules with chemical challenges require tailor solutions for efficient drug capture and delivery. On the application end, some require high systemic drug levels, while others require pulsed delivery or delivery to specific cell types. Certain drug molecules require rapid uptake to enhance the effectiveness and/or to combat the instability. Still, others require prolonged release of low levels of drug. The efficacy of many drugs is often limited by their potential to reach the site of action. In most cases, only a small amount of administered dose reaches the target site, therefore, developing a drug delivery system that optimizes the pharmaceutical action of a drug, while reducing its toxic side effects is a challenging task. Moreover, particulate drug delivery system offers a number of advantages over conventional dosage form. Among the various drug delivery system investigated, nanoparticles represents a very promising approach to achieve the capability of delivering drugs to target areas.

Nanotechnology is the creation of functional materials, devices and systems, through the understanding and control of matter at dimensions in the nanometer
scale length (1-100 nm), where, new functionalities and properties of matter are observed and harnessed for a broad range of applications. Nanotechnology has impact on a number of areas in the Life Sciences – from cosmetics and personal care to diagnostics, therapeutics, and medical technology. Nanotechnology provide innovations in various fields of medicine namely therapy, diagnostic, imaging and drug delivery (Barratt, 2000, Freitas, 2005 and Sonvico et al., 2005). Nanoparticles are unique drug delivery system, which enhances the bioavailability of drugs by providing controlled drug delivery. Nanoparticles provide effective and patient compliance alternative to conventional drug delivery. Since nanoparticles are very small, they have the ability to cross the biological barriers and reach the target area.

Based on the type of the inactive ingredient used, four classes of nanoparticles for drug delivery (Sharma et al., 2013) have been devised, viz., Metal-based nanoparticles; Lipid-based nanoparticles; Polymer-based nanoparticles and Biological nanoparticles.

1.8.1 Metal based nanoparticles

Metal nanoparticles have attracted much attention in the fields of physics, chemistry, electronics and biology (Henglein et al., 1989, Schmid, 2008 and Lanje et al., 2010) because of the unique electrical (Peto et al., 2002), chemical (Kumar et al., 2003), optical (Królikowska et al., 2003) and photo electrochemical (Chandrasekharan et al., 2000) properties, which are strongly dependent on the size and shape of the metal nanomaterials (AlanáCreighton , 1991, Liu et al., 1995 and Zhang et al., 2000 ). Metal nanoparticles have a high specific surface area and a high surface to volume ratio. Metal nanoparticles are particularly interesting systems
because of the ease with which they can be synthesized and modified chemically (Chou et al., 2000 and Ying et al., 2005).

Use of polymer as a carrier for metal based nanoparticles that can provide targeted drug delivery combined with optimal drug release profile (Tiyabooncha, 2013). Recently, the use of nanoparticles made up of chitosan has drawn much interest due to their association and delivery of variety of drug moieties.

Based on the descriptions on diabetes, types, treatments, remedy through herbal medicines, importance of trace elements and novel delivery system based on nanotechnology, in the present study, an approach was made using a plant active constituent as an anti-diabetic agent. Further, it comprises, complexation with transition metals and transform into a nanoparticles. Furthermore, it involves evaluation of the efficacy of the resultant product under \textit{in vivo} conditions and \textit{in silico} model system to substantiate further.

Thus, the present study has been described in the form of six major chapters and the following paragraph emphasizes the major objectives and the outline of the chapters.

1.9 \textbf{Major objectives of the present study}

- Screening and identification of natural plant quinones
- Extraction and purification of active constituent of the chosen herbal plant
- Preparation of transition metal complexes with the active constituent
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- Preparation of nanoparticles of metal complexes of active constituent for effective delivery
- Evaluation of the antidiabetic activity of the nanoparticles under *in silico* methods
- Evaluation of antidiabetic activity of the nanoparticles of the active constituent and its metal complexes under *in vivo* methods

Structure of the Thesis

In accordance with the aim and objectives of the study, the chapter of the thesis has been structured as follows:

Chapter I  – Introduction

This chapter mainly focuses on general information on diabetes, types, treatment methods, use of herbal products, selection of plant, active constituents, importance of transition metals, metal complexes, nanoparticles, delivery systems, need for the study, objectives of the study and finally the structure of the study.

Chapter II  – Review of Literature

This chapter describes the systematic literature review analyzed with regard to diabetes, treatment methods, embelin, pharmacological activity related to embelin and nanoparticles.

Chapter III  – Extraction, purification, preparation and characterization of embelin and its metal complexes

This chapter detailed the procedure and methods followed for the extraction of embelin and preparation of its complexes followed by the instrumental analyses
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for the identification of natural benzoquinone and its metal complexes like copper and zinc.

Chapter IV – Preparation and characterization of nanoparticles of Embelin and its metal complexes

In this chapter, nanoparticle preparation of embelin and its metal complexes and its characterization were described in detail.

Chapter V – Evaluation of anti-diabetic efficacy of the nanoparticles of embelin and its metal complexes

This chapter has been divided into two sub chapters, namely, Chapter Va and Chapter Vb.

Chapter Va – In silico assessment on antidiabetic efficacy of embelin and its metal complexes

In this chapter, in silico model approach has been adopted to evaluate the anti-diabetic efficacy of embelin and its metal complexes using enzyme based system and the observations and results were discussed in detail.

Chapter Vb – In vivo studies on antidiabetic efficacy of embelin and its metal complexes

This chapter describes the evaluation of antidiabetic efficacy of nanoparticles of embelin and its metal complexes in diabetic induced animal models. Evaluation of biochemical profile of blood serum and urine and sectioning and staining of major organelles done to understand the antidiabetic potential of the chosen nanoparticles.
Chapter VI  – Summary and Conclusion

The final section of the thesis mainly emphasizes the major outcome from each chapter and the final conclusion made from the study. It also discusses path forward and the avenues for future research.

References will be included at the end of the each chapter of the thesis.

Finally, the publications made from the observations of the present study have been enclosed herewith.
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http://www.idf.org/signs-and-symptoms-diabetes