INTRODUCTION
2. INTRODUCTION

Diabetes mellitus is a common and prevalent disease affecting the citizens of both developed and developing countries. It is a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. It is the single most important metabolic disease, which can affect nearly every organ system in the body. According to the recent data from World Health Organization (WHO) and International Diabetes Federation (IDF), the number of people affected with diabetes worldwide has increased dramatically over recent years. Currently there are over 366 million diabetics worldwide and this is likely to increase to 552 million more by the year 2030 (Whiting et al, 2011; WHO, 2011). The estimated numbers of adults with diabetes in India is expected to be 87 million in 2030 (Ramachandran et al, 2010). India was home to 62.4 million diabetics according to International Diabetes Federation in 2011(Venkatachalam et al, 2012). The reasons for this escalation are due to changes in lifestyle; people living longer than before (ageing) and low birth weight could lead to diabetes during adulthood. The disease becomes a real problem to public health in developing countries, where its prevalence is increasing steadily and adequate treatment is often expensive or unavailable.

Hyperglycemia is involved in the etiology of development of diabetic complications. Chronic hyperglycemia often leads to microvascular complications that include nephropathy, retinopathy, neuropathy and macrovascular complications that include coronary artery disease, leading to myocardial infarction (heart attack) or angina, stroke (mainly ischemic type), peripheral vascular disease, which contributes to intermittent claudication (exertion-related foot pain) as well as diabetic foot. All these complications lead to significant morbidity and mortality in patients with diabetes (Fowler, 2008). Diabetic patients
display a markedly greater incidence of cardiovascular disease as compared with the non-diabetic population. Diabetic vascular complications cause acquired blindness, end-stage renal failure, a variety of neuropathies and atherosclerosis in diabetic patients (Lebovitz, 1984; Fein and Sonnenblick, 1985). On the other hand, cardiac diastolic dysfunction is the primary heart defect in diabetes and is manifested as impaired diastolic performance and depressed systolic function (Fein and Sonnenblick, 1985; Ren and Ceylan-Isik, 2004). Diabetic heart dysfunction, namely diabetic cardiomyopathy, occurs independent of the macro- and micro-coronary vascular diseases (Fein and Sonnenblick, 1985). Diabetic cardiomyopathy is characterized by reduced wall compliance and rate of myocardial relaxation (Fein and Sonnenblick, 1994; Ren and Davidoff, 1997; Ren and Ceylan-Isik, 2004). There also occurs functional changes such as shortened left ventricular ejection time, increased pre-ejection period and increased wall stiffness, decreased fractional shortening, rate of left ventricular filling and increased action potential duration in diabetic hearts (Buyukgebiz et al, 2000; Poirier et al, 2001; Li et al, 2006).

A yet another frequent complication that occurs in both type 1 and type 2 diabetes is diabetic retinopathy. It is considered to be fifth most common cause of blindness in the United States (Klein and Klein, 1997). In 95% of type 1 diabetics and 60% of type 2 diabetics with disease duration longer than 20 years, signs of diabetic retinopathy occur. More severe cases of proliferative diabetic retinopathy are seen in patients suffering from type 1 diabetes. Tight control of hyperglycemia, blood lipids, and blood pressure has been shown to be beneficial to prevent its development or progression (Guillausseau et al, 1998; Turner, 1998; Stratton et al, 2001).

Cataract is considered to be a major cause of visual impairment in diabetic patients as the incidence and progression of cataract is elevated in patients with diabetes mellitus (Kahn et al, 1977; Harding et al, 1993). The association between diabetes and cataract formation has been shown in clinical epidemiological and basic research studies. Due to increasing numbers of type 1
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and type 2 diabetics worldwide, the incidence of diabetic cataracts has been rising steadily.

During hyperglycemia, extra cellular glucose diffuses into the lens, which can lead to post-translational modification. The cataractogenesis is mainly due to synthesis and accumulation of excessive sorbitol in the lens fibers and consequent osmotic stress. Sorbitol is synthesized by aldose reductase utilizing NADPH and does not easily cross cell membranes. It accumulates in cells and causes damage by disturbing osmotic homeostasis (Gupta et al, 2009). Glucose autoxidation, formation of AGE, and activation of the polyol pathway cause intracellular accumulation of sorbitol, which causes various types of ocular lesions, alterations on the membrane permeability, loss of glutathione and a diminution of the protein synthesis. Aldose reductase, key enzyme of polyol pathway, catalyzes the reduction of glucose into sorbitol, which is subsequently metabolized into fructose by sorbitol dehydrogenase. Sorbitol, an osmolyte leads to osmotic swelling, changes in membrane permeability, leakage of glutathione and myo-inositol and perhaps even the generation of free radicals and hydrogen peroxide, primarily causing for the development of diabetic complications such as cataract, retinopathy, neuropathy and nephropathy (Jung et al, 2011). Another pathophysiological mechanism involved in the formation of cataract is deficient glutathione (GSH) levels because of which lens proteins remain in their reduced form. Oxidative stress is also associated with development of cataract. Diabetes causes increased levels of oxidized DNA, proteins, and lipids, that contribute to various diabetic complications (Suryanarayana et al, 2005). Currently, the only available treatment for the disease is the surgical extraction of the cataractous lens followed by replacement with a synthetic implant (Javadzadeh et al, 2009). Even though cataract surgery is an effective cure, there is a need to develop drugs to delay or prevent the development of cataract in diabetic patients because patients with diabetes mellitus have higher complication rates from cataract surgery (Stanga et al, 1999).

Conventionally, insulin-dependent diabetes mellitus is treated with exogenous insulin (Felig et al, 1995) and non insulin-dependent diabetes mellitus
with synthetic oral hypoglycemic agents like sulphonylureas and biguanides (Rosac, 2002). These drugs have prominent side effects and fail to significantly alter the course of diabetic complications. As the knowledge of heterogeneity of this disorder increases, there is a need to look for more efficacious agents with lesser side effects. Though development of modern medicine has resulted in the advent of successful drugs like insulin, biguanides, sulfonylureas and thiazolidinediones, there is still a need to look for new drugs because no drug (except strict glycemic control with insulin) has been shown to modify the course of diabetic complications.

Herbal medicines are the medicinal products that contain plant materials as their pharmacologically active components (Schulz et al., 1998). Medicinal plants are important for pharmacological research and drug development, not only when plant constituents are used directly as therapeutic agents, but also as starting materials for the synthesis of drugs or as models for pharmacologically active compounds. Herbal drugs are of three types based on the nature of the active metabolites. Drugs used in crude form are the first category. The active constituents isolated after the processing of plant extracts represent the second category of herbal drugs. These are pure molecules and generally pharmacologically more active. Herbal drugs for which data on acute and chronic toxicity studies in animals is available represent the third type (Iwu et al., 1999). It is estimated that about 25% of the drugs prescribed worldwide are derived from plants and 121 such active compounds are in use. The World Health Organization (WHO) has listed 21,000 plants, which are used for medicinal purposes around the world. Nearly 80% of African and Asian population depends on traditional medicines for their primary healthcare (WHO website, 2008).

India has an ancient heritage of traditional medicine. Materia medica of India provides lots of information on the folklore practices and traditional aspects of therapeutically important natural products. Indian traditional medicine is based on various system including Ayurveda, Siddha and Unani. Also China and UK have also got their own traditional system of medicine. These traditional systems have their uniqueness but there is a common thread running through these
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systems in their fundamental principles and practices. With the emerging interest in the world to adopt and study the traditional system and to exploit their potentials based on different healthcare systems, the evaluation of the rich heritage of the traditional medicine is essential. India is the largest producer of medicinal herbs and is called as botanical garden of the world (Seth and Sharma, 2004). In India, about 80% of the rural population uses medicinal herbs or indigenous systems of medicine (Mukherjee and Wahile, 2006). India has about 45,000 plant species and several thousands have been claimed to possess medicinal properties (Grover et al, 2002). Plants are source of extremely wide range of chemical components. At least hundred chemical substance of known structure are extracted from plants that are being used as drugs throughout the world. In many countries it is traditional to use medicinal plants, either a single herb or a polyherbal formulations, to control diabetes. The antihyperglycaemic effect of several plants extracts or herbal formulation that are used as a antidiabetic remedies has been confirmed (Sharma et al, 1992).

Different medicinal systems are using the active plant constituents, which discovered as natural hypoglycemic medicine, came from the virtue of traditional knowledge. Herbal drugs are considered free from side effects than synthetic one. Many active compounds have been isolated from the plant and herb species of India. These active principles are dietary fibres, alkaloids, flavonoids, saponins, amino acids, steroids, peptides and others. These have produced potent hypoglycemic, anti-hyperglycemic and glucose suppressive activities (Saxena et al, 2006). The above effects are achieved by either insulin release from pancreatic β-cells, inhibited glucose absorption in gut, stimulated glycogenesis in liver or increased glucose utilization by the body (Grover et al, 2002; Saxena and Vikram, 2004). These compounds also exhibited antioxidant, hypolipidemic and anticataract activities; restored enzymatic functions, repair and regeneration of pancreatic islets and the alleviation of liver and renal damage (Mukherjee et al, 2006). Some active constituents obtained from plants possess insulin like activity and could provide alternate for insulin therapy.
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Hundreds of products are marketed in India as "natural" agents for lowering blood sugar and decreasing long term complications. These include Alfabetic, Diabetic, Diabets, DB-7, Diabetica, Diabetiks, Dia-Comp, DiaVite, GlucoCare, Glucotize, GlycoNase, SugarMax, and Sugar Loss. These formulations are typically the combination products containing the individual components presented here along with others. Various cultures have remedies unique to their own population, such as neem (Azadirachta indica) in India (Chattopadhyay 1996), Pedra hume caa (Myrica uniflora) in Brazil (Russo et al., 1990), tronadora (Tecoma stans) in Mexico (Lozoyz-Meckes, 1985). In relation to plants also, barring a few studies (Rathi et al, 2002; Grover et al, 2000; Karunanayake et al, 1990; Srivastava et al, 1988), most of the studies have not assessed the impact of these plants on the course of diabetic complications. Traditional plant medicines or herbal formulations might offer a natural key to unlock diabetic complications (Nammi et al, 2003).

_Tephrosia purpurea_ (Linn.) pers, is a polymorphic, much-branched suberect perennial widely growing herb belonging to _Fabaceae_ (Leguminosae) family. The roots, leaves, seeds, and bark were used medicinally. The herb has white to purplish flowers and can be found in tropical regions. It is common in India, Sri Lanka, Malay Peninsula, China and Hawaii. It is popularly known as “Sarapunkha” in Sanskrit, “Unhall” in Gujarati, “Purpule tephrosia” in English and “Kattukkolinca” in Tamil (Kirtikar and Basu, 1975; Anonymous, 1976; Nadkarni 1989). According to _Ayurveda_, _T.purpurea_ is used as digestible, anthelmintics, alyseretic, antipyretic, astringent, thermogenic, acrid and also used to cure diseases of liver, spleen, heart, blood, tumours, ulcers, leprosy and asthma (Warrier et al, 2004). Unani system of medicine describes the roots as diuretic, allays thirst, enriches blood, cures diarrhea, useful in bronchitis, liver, inflammations, boils and pimples (Bhandari, 1949).

Phytochemical investigations of _T. purpurea_ have revealed the presence of glycosides, rotenoids, isoflavones, flavanones, chalcones, flavanols, flavones and sterols (Pelter et al, 1981). Rutin is a flavonol type of flavonoid present in about 4.65% in the leaves of _T.purpurea_ (Prashanth Kumar et al, 2003). The
plant is reported to contain sitosterol, lupeol, rutin chloride, isolanchocarpin, lanceolatin A, pongamol, karangin, 5,7 dimethoxy-8- flacanone, 2-methoxy 3,9-difyarosy coumestone, flavochapparins B and C, methyl karangic acid and purpurin (Siddiqui et al, 2009). It is also reported to contain three novel flavonoids, (+)-tephrorins A and B and (+)-tephrosone, an isoflavone, 7,4'-dihydroxy-3',5'-dimethoxyisoflavone and a chalcone, (+)-tephropurpurin (Chang et al, 1997; Chang et al, 2000).

Different parts of the plant have been extensively studied for various pharmacological actions. The dried herb is effective as tonic laxative, diuretics and deobstruents. It is also used in the treatment of bronchitis, bilious febrile attack, boils, pimples and bleeding piles. The roots and seeds are reported to have insecticidal and piscicidal properties and used as vermifuge. The roots are also reported to be effective in leprous wound and their juice, to the eruption on skin. An extract of pods is effective for pain, inflammation and their decoction is used in vomiting (Anonymous, 1976). The aqueous extract of seeds has shown significant in vivo hypoglycaemic activity in diabetic rabbits (Rahman et al, 1985). The aqueous and ethanolic seed extract has shown potent antihyperglycemic activity in streptozotocin diabetic rats (Pavana et al, 2007a; Pavana et al, 2009). T. purpurea leaf extract have also been demonstrated to have anti-hyperglycemic activity (Pavana et al, 2007b). Further, Joshi et al (2008) proved the hypoglycemic activity of the root extracts in normal and alloxan diabetic rats. Effect of the plant on the long term diabetic complications has not been studied so far and moreover, there is no standard treatment available for the therapy of diabetic complications. Thus, the aim of this study was to investigate the effect of the aqueous extract of the whole plant on streptozotocin induced diabetes and its related complications such as cardiovascular complications and cataract.

Phytochemical evaluation is one of the tools for the quality assessment, which includes preliminary phytochemical screening, chemoprofiling and marker compound analysis using modern analytical techniques like HPTLC and HPLC. Although several formulations available for diabetes, very limited attempts have
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been made to evaluate their pharmacological aspects and verify therapeutic efficacy. Moreover, because of resurgence of interest on herbal drugs, it is also important to ensure that only quality products enter the market. Efforts are being made by various government agencies and research laboratories to maintain the quality of herbal drugs by proper identification and detailed pharmacognostic, phytochemical investigations and standardization. However, in spite of the continuing efforts, there are no standard methods available for quality control of herbal drugs, which is the main hurdle for India to enter into the multi-million dollar international market. Further, the composition of plant material can vary and it is known to be influenced by the place of origin, soil, climate, season, time of collection, post harvesting conditions, temperature changes, moisture which affect tremendously the quality and therapeutic efficacy of the drug. Therefore, the quality and efficacy of the herbal drugs need to be established through systematic pharmacognostic, phytochemical and pharmacological evaluation and standardization of the drug. In herbal research, it is also essential to authenticate the plant and to establish phytochemical standardization with help of reliable instruments like HPTLC. Before undertaking pharmacological work, we also carried out pharmacognostic and phytochemical standardization of *T. purpurea* extract and its flavonoidal fraction using HPTLC fingerprinting.