1. INTRODUCTION

Diabetes mellitus can be described as a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism due to defects in insulin secretion, insulin action, or both (Craig et al., 2009). According to ancient Hindu physicians, ‘Madhumeha’ is a disease in which a patient passes sweet urine and exhibits sweetness all over the body. They had recorded in their observations that ‘if too many ants swarm around a spot of urine, then the person have symptoms of diabetes mellitus (Warjeet Singh, 2011). The most recent statistics indicate that the global prevalence of diabetes mellitus, estimated as 366 million in 2011, will increase to 522 million by 2030 (Whiting et al., 2011). The current studies in India indicate that there is an alarming rise in prevalence of diabetes which has gone beyond epidemic form to a pandemic one (Meenakshi et al., 2010; Pareek et al., 2009). Globally, diabetes mellitus presents enormous and increasingly important public health issues. The occurrence and consequences associated with diabetes are found to be high in countries like India (31.7%), China (20.8%) and United State of America (17.7%) (Balaraman et al., 2010). It is predicted that by 2030, India, China and the United States will have the largest number of people with diabetes (Frode and Medeiros, 2008).

The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels. Symptoms of marked hyperglycemia include polyuria, polydipsia, and weight loss, sometimes with polyphagia and blurred vision. Impairment of growth and susceptibility to certain infections may also accompany with chronic hyperglycemia. Patients with diabetes have an increased incidence of atherosclerotic cardiovascular, peripheral arterial and cerebrovascular disease. Hypertension and abnormalities of lipoprotein metabolism are often found in people with diabetes (ADA, 2010). Mortality and morbidity associated with diabetes is mainly due to complications arising from it which include neuropathy, nephropathy and retinopathy (Amos et al., 1997).
1.1 Type of diabetes mellitus

Based upon the etiology, diabetes mellitus can be divided into two main types, Type 1, “Juvenile Diabetes Mellitus” (Insulin Dependent Diabetes Mellitus) and Type 2, “Adult type” (Non-Insulin Dependent Diabetes Mellitus) and Other type of diabetes is gestational diabetes(Thevenod and Warjeet Singh, 2008). In most western countries, type 1 diabetes accounts for over 90% of childhood and adolescent diabetes although less than half of individuals with type 1 diabetes are diagnosed before the age of 15 years. Type 2 diabetes is becoming more common in youth onset diabetes in certain at risk populations. In addition, there is a distinct slowly progressive form of type 1 diabetes in Japan, which represents approximately one third of cases of type 1 diabetes. Type 1 diabetes is more common in the offspring of diabetic men compared with diabetic women (Craig et al., 2009).

1.1.1 Type I Diabetes

Type I diabetes is an autoimmune disease. An autoimmune disease results when the body’s system for fighting infection (the immune system) turns against a part of the body. In diabetes, the immune system attacks and destroys the insulin-producing beta cells in the pancreas. The pancreas then produces little or no insulin. At present, scientists do not know exactly what causes the body’s immune system to attack the beta cells, but they believe that autoimmune, genetic, and environmental factors, possibly viruses, are involved. It develops most often in children and young adults but can appear at any age. Symptoms of type I diabetes usually develop over a short period, although beta cell destruction can begin years earlier. Symptoms may include increased thirst and urination, constant hunger, weight loss, blurred vision, and extreme fatigue. If not diagnosed and treated with insulin, a person with type I diabetes can lapse into a life-threatening diabetic coma, also known as diabetic ketoacidosis (Craig et al., 2009).

1.1.2 Type II diabetes

Type II diabetes mellitus is an increasingly common disorder of carbohydrate and lipid metabolism two important characteristics of this disease are insulin resistance, the failure of peripheral tissues; including liver, muscle, and adipose tissue, to respond to physiologic doses of insulin, and failure of pancreatic cells to properly secrete insulin in response to elevated blood glucose levels. Obesity is a significant
risk factor for the development of type II diabetes mellitus. An extremely lean and lipoatrophic models have revealed a similar predisposition to developing diabetes. Although it may seem paradoxical that both increased adiposity and severely reduced fat mass cause diabetes, a common pathophysiologic process in fat may be responsible for the predisposition to develop hyperglycemia in both conditions (Nadler and Attie, 2001).

Broadhurst proposed the major causative factors for Non-Insulin Dependent-Diabetes Mellitus (NIDDM) involving obesity and over fatness; carbohydrate and fat over nutrition; lack of polyunsaturated fatty acids (PUFA) in plasma membranes and unbalanced triglyceride intake; chromium deficiency; and lack of soluble fiber and relevant beneficial phytochemicals (Broadhurst et al., 1997).

NIDDM is a complex disease that is currently thought to be influenced by more than a single gene or environmental factor. Although the relative contribution of genetic and environmental factors to the development of NIDDM differs among individuals, patients generally have two common metabolic abnormalities: insulin resistance and defects in glucose-stimulated insulin secretion, which lead to disease state.

1.1.3 Gestational Diabetes

Some women develop gestational diabetes late in pregnancy. Although this form of diabetes usually disappears after the birth of the baby, women who have had gestational diabetes have a 20 to 50% chance of developing type II diabetes within 5 to 10 years. Maintaining a reasonable body weight and being physically active may help prevent development of type 2 diabetes. As with type 2 diabetes, gestational diabetes occurs more often in some ethnic groups and among women with a family history of diabetes. Gestational diabetes is caused by the hormones of pregnancy or a shortage of insulin. Women with gestational diabetes may not experience any symptoms (Craig et al., 2009).

1.2 Pathophysiology of diabetes mellitus

Diabetes mellitus has a profound adverse effect on quality of life in terms of social, psychological well-being as well as physical health. Diabetic complications are
mainly mediated through oxidative stress such as increased production of ROS or impaired antioxidant defense systems. Oxidative stress can be defined as a state of imbalance toward the factors that generate reactive oxygen radicals (e.g., superoxide or hydroxyl radicals) and away from the factors that protect cellular macromolecules from these reactants including antioxidants like superoxide dismutases, catalase, and glutathione peroxidases. The factors that generate reactive oxygen species (ROS) exist as products of normal cellular physiology as well as from various exogenous sources. Mitochondria are thought to be the source of most cellular ROS, specifically superoxide radicals. The reactions that generate ATP in the mitochondria require electrons from reduced substrates to be passed along the complexes of the electron transport chain. In the presence of molecular oxygen, electrons that leak from this process react and form the free radical superoxide. Superoxide anions are significant mediators in numerous oxidative chain reactions and are also a precursor to many other ROS (Halliwell and Gutteridge, 1989).

Enhancement of lipid peroxidation, alteration in antioxidant enzymes and impaired glutathione metabolism are the main factors involved in the development of diabetes (Dewanjee et al., 2009). Production of free radicals is also involved in the pathogenesis of various type of disease including diabetes mellitus (Bagri et al., 2009). Increased formation and accumulation of advanced glycation products (AGEs) is also involved in the diabetic complications such as retinopathy, neuropathy, and renal dysfunction through a series of pathological changes (Ding et al., 2010). Though several hormones are involved in the regulation of blood glucose level, the most important ones are insulin and glucagon.

When imbalanced occurs in the level of hormones in the body, sugar starts accumulating in the blood and when concentration of glucose increased in the blood then finally it will passes in urine along with other minerals (Warjeet Singh, 2011 ). In most cases of diabetes, primarily T-cell mediates pancreatic islet-cell destruction, and becomes clinically symptomatic when 90% of pancreatic beta cells are destroyed. Serological markers such as islet cell, glutamic acid decarboxylase (GAD), IA-2, IA-2., or insulin autoantibodies, are present in 85-90% of individuals when fasting hyperglycemia is detected. Sometimes environmental triggers, such as chemical or viral initiated pancreatic β-cell destruction, which can trigger consequences and
thereby leads to the cause in diabetes mellitus. From the study it was found that enterovirus infection is also associated with the development of diabetes mellitus (Craig et al., 2009).

1.3 Diabetic and oxidative stress

Oxidative stress plays a pivotal role in the development of diabetes complications, both microvascular and cardiovascular. During diabetes, persistent hyperglycemia causes increased production of free radicals especially reactive oxygen species (ROS), for all tissues from glucose auto-oxidation and protein glycosylation (West, 2000). Free radicals are generated as by-products of normal cellular metabolism; however, several conditions are known to disturb the balance between ROS production and cellular defense mechanisms. This imbalance can result in cell dysfunction and destruction resulting in tissue injury. The increase in the level of ROS in diabetes could be due to their increased production and/ or decreased destruction by non-enzymic and enzymic catalase (CAT), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) antioxidants. The level of these antioxidant enzymes critically influences the susceptibility of various tissues to oxidative stress and is associated with the development of complications in diabetes. Also this is particularly relevant and dangerous for the beta islet, which is among those tissues that have the lowest levels of intrinsic antioxidant defenses (Lenzen et al., 1996; Robertson, 2004).

1.4 Diabetes and Hypertension

Hypertension is an extremely common co-morbidity of diabetes. From past two decades, hypertension and diabetes are the chief diseases occurring to majority of people throughout the world. Hypertension is seen in approximately 30.5% of population i.e, 35 out of 100 will suffer with diabetes up to 2025. Increase in the rate of the blood flow in the blood vessels is called as hypertension. It is of two types; transient and persistant. Transient is seen during the emotional upset or physical exertion or during fever conditions. And persistant is due to various other factors. Blood pressure is defined as product of cardiac output (CO) & total pheripheral resistance (TPR). Vasconstriction arised by sympathetic nervous system is main factor that increases TPR. Hypertension is the condition where the systolic pressure is greater than 140 mm Hg & diastolic pressure greater than 90 mm Hg (Senthil et al., 2012).
1.5 Angiotensin I–converting enzyme (ACE) Inhibitor

Angiotensin I–converting enzyme (ACE) is a key enzyme that regulates blood pressure via renin-angiotensin system (RAS) (Chen, et al., 2009; Erdmann, et al., 2008). It catalyzes the degradation of the decapeptide angiotensin I to the potent vasoconstrictor angiotensin II by cleavage of dipeptides at carboxyl-terminal sites. It is also able to degrade bradykinin, a vasodilator, into inactive peptides (Skeggs, et al., 1956; Yang and Erdos, 1967; Yang, et al., 1970). This dual role enables activation of ACE on the overall elevation of blood pressure. There are available synthetic ACE inhibitors, the uses of which have achieved great success. For instance, captopril was the first ACE inhibitor successfully used in clinical practice (Ondetti, et al., 1977). However, captopril and the other known synthetics, have significant adverse effects such as cough, exanthema, taste alterations, skin rashes, gastric troubles and edema of lips (Vyssoulis, et al., 2001; Torruco-Uco, et al., 2009). Because of the adverse side effects of the synthetic compounds, there is intense interest in the extraction of ACE inhibitors from natural food sources to serve as substitutes or replacements which could curtail the reliance on synthetic compounds. As a result, peptides with ACE inhibitor activities have been characterized from several natural sources, for example, milk (Contreras, et al., 2009; Chen, et al., 2007; Jiang, et al., 2007), soy (Lo & Li-Chan, 2005; Mallikarjun Gouda, et al., 2006; Wu & Ding, 2002), sunflower (Megías et al., 2004), beef (Jang & Lee, 2005), buckwheat (Ma, et al., 2006), shark meat (Wu, et al., 2008) and peanuts (Quist, et al., 2009). Several studies have been reported on ACE inhibitory peptides from pulses, such as chickpea (Barbana and Boye, 2010; Pedroche, et al., 2002; Yust, etal, 2003), pea (Barbana & Boye, 2010; Vermeirssen, et al., 2004; Vermeirssen, et al., 2005), lentil (Boye, et al., 2010; Barbana & Boye, 2011).

In India, 23.10 % men and 22.60 % women over 25 years old suffer from hypertension, says the World Health Organisation’s ‘global health statistics 2012’ released in May, 2012. Recent studies show that for every known person with hypertension in India, there may possibly be 2 persons with either undiagnosed hypertension or prehypertension. With over 139 million patients, India accounts for 15 % of world’s uncontrolled hypertension patients A survey of 26,000 adults in South India showed a hypertension prevalence of 20% (Men 23% and Women 17%). 67% of people suspected by hypertension but unaware of their diagnosis. Inida’s largest
a clinic-based survey study called Screening India’s Twin Epidemic (SITE) showed that 1 in every 5 India adults living in urban cities suffered both hypertension and diabetes.

Hypertension is currently the leading risk resulting in considerable death and disability worldwide and accounted for 9.4 million deaths and 7 per cent of disability adjusted life years in 2010 (Lim et al., 2013). Hypertension is increasing rapidly in most low and middle income countries driven by diverse health transitions. In India, hypertension is the leading non-communicable disease (NCD) risk and estimated for nearly 10 per cent of all deaths (Patel et al., 2011). Adult hypertension prevalence has risen dramatically over the past three decades from 5 per cent to between 20-40 per cent in urban areas and 12-17 per cent in rural areas (Gupta et al., 2004 & Reddy et al., 2005). The number of hypertensive individuals is anticipated to nearly double from 118 million in 2000 to 213 million by 2025 (Reddy et al., 2000). It is estimated that 16 per cent of ischaemic heart disease, 21 per cent of peripheral vascular disease, 24 per cent of acute myocardial infarctions and 29 per cent of strokes are attributable to hypertension underlining the huge impact effective. Hypertension prevention and control can have on reducing the rising burden of cardiovascular disease (CVD) (Mohan S et al., 2011).

Twenty per cent of India’s population preferable the age of 30 years suffer from diabetes and high blood pressure. Over 4 crore people from across the country screened under the Government’s National Programme for Prevention and Control of Cancer, Diabetes, Cardiovascular Diseases and stroke (NPCDCS) has revealed that 6.34 per cent of the population is suspected to be suffering from diabetes and over 6 per cent are hypertensive. Unless we take medical awareness against high BP and diabetes will continue to take a high toll of our population. It leads to heart attacks, brain strokes, chronic kidney and early blindness and is preventable by taking preventive measures early in life. The survey results in urban areas of the country including Delhi, Bangalore, Ahmedabad, Chennai and Kamrup (Assam) has pointed out trends that almost 11 per cent people are suspected to be suffering from diabetes and 13 per cent are hypertensive.
1.6. Antioxidants

Antioxidants are the compounds of exogenous or endogenous in nature which either prevent the generation of toxic oxidants or intercept any that are generated and inactivate them and thereby block the propagation of chain reaction produced by these oxidants (Willcox, 2004). These can be classified as enzymatic antioxidants as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and non-enzymatic antioxidants like (nutrients antioxidants) beta-carotene, α-folopherol ascorbic acid, bioflavonoid and (metabolic antioxidants) like glutathione, ceruloplasmin, albumin, bilirubin, ferritin, transferring, uric acid and lactoferrin. In recent years increasing experimental and clinical data has provided compelling evidences for the involvement of antioxidant in the treatment of various free radical mediated diseases including diabetics (Velavan, 2011; Halliwel, 1994).

1.7. Medicinal plants

Medicinal plants are assuming greater importance in the primary health care of individuals and communities in many developing countries. There has been an increase of demand in international trade because of very effective, cheaply available, supposedly have less or no side effects and used as alternative to allopathic medicines. Medicinal plants are believed to be much safer and proved elixir in the treatment of various ailments (Ashis, 2003). In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. Medicinal plants are a major source of biodynamic compounds of therapeutic values (Harsha et al., 2002).

There is a resurgence of interest in herbal medicine for the treatment of various ailments, chiefly because of the prohibitive cost of allopathic drugs, their unavailability in remote areas and the popular belief that naturally occurring products are without any adverse side effects. Various spices and herbs used in the traditional Indian System of medicine, ‘Ayurveda’, have anti-thrombotic, cardioprotective, anti-atheroscleortic, hypoglycemic, (hypolipidemic, anti-inflammatory and anti-arhtritic) properties (Viswanatha et al., 2010; Tilak-Jain and Devasagayam, 2006).
1.8. BRASSICA OLERACEA L.

1.8.1 Scientific classification

Kingdom : Plantae
Order : Brassicales
Family : Brassicaceae
Genus : Brassica
Species : B. oleracea

1.8.2. Vernacular Name

English : Cabbage
Tamil : Muttaikkos
Hindi : Bantgopl
Malayalam : Mottakkusu
Telungu : Kosugadda

1.8.3. Description:

A suffruticose biennial 0.5 m in height, stems quite glabrous branching in the upper part leaves basal and lower cauline large, stalked, nerves stout, white, especially prominent below, margins crenulate and undulate, the middle cauline leaves ampelxical oblong–obovate, obtuse, the upper leaves oblong-linear, almost entire, all leaves fleshy, glabrous; flowers large pale yellow, 20-40 in racemes; fruits irregularly spreading or ascending linear pods, tetragonous, often serpentine flexuose with a beak, mostly one-seeded, seeds globose, obscurely brown, pendulous.

Habitat: cultivated on hill-tops of India
Propagation: By Seeds
Parts used: leaves

1.8.4 Chemical Constituents: The essential amino acid composition of turnip greens. The leaf protein concentrates are rich in lysine and tryptophan and are a good supplement to food deficient in these amino acids dehydrated turnip root meal.

Uses: Leaves are sweet, cooling, stomachic, anti scurbutic, emollient, constipating diuretic anthelmintic and cardiotonic. They are useful in abdominal disorders pruritus,
skin diseases, diarrhoea, strangury, intestinal worms, cough bronchial asthma, fever, warts, urorrea, haemorrhoids, gout and vitiated conditions of pitta and vata (Prajapati et al., 2006).

1.8.5 Nutritional composition

The nutritional health and well-being of humans are entirely dependent on plant based food, as plants are critical components of the dietary food chain. Plants are a good source of health-promoting chemicals, providing almost all essential mineral and organic nutrients to humans either directly, or indirectly. Brassica vegetables are rich source of bioactive compounds. *Brassicaceae* vegetables are considered to be a food in many areas all over the world. *Brassicaceae* vegetables are also recognized as a rich source of nutrients such as vitamins carotenoids, tocopherol, ascorbic acid, folic acid, minerals Cu, Zn, P, Mg among others, carbohydrates sucrose and glucose, amino acids L-alanine, L-aspartic acid, L-glutamic acid, L-glutamine, L-histidine, L-methionine, L-phenylalanine, L-threonine, L-tryptophan, and L-valine, fibers, and different groups of phytochemicals such as indole phytoalexins brassinin, spirobrassinin, brassilexin, camalexin, 1-methoxyspirobrassinin, 1-methoxyspirobrassinol, and methoxyspirobrassinol methyl ether, phenolic such as feruloyl and isoferuloylcholine, and hydroxybenzoic, neochlorogenic, chlorogenic, caffeic, p-coumaric, ferulic, and sinapic acids, anthocyanins, quercetin and kaempferol, and glucosinolates mainly including glucoiberin, glucoraphanin, glucoalyssin, gluconapin, glucobrassicanapin, glucobrassicin, gluconasturtiin, and neoglucobrassicin. All these phytochemicals significant activities reported as antioxidant, antidiabetics, anticarcinogenic, and cardiovascular diseases.
Figure 1. *Brassica oleracea* L.
OBJECTIVES OF THE STUDY

The following are the main objectives of the present study

1. Preparation of aqueous and solvent extract of red cabbage to investigate its anti diabetic activity, antioxidant activity, angiotensin I converting enzyme inhibitor activity.

2. To study changes in enzymes activity involved in carbohydrate and lipid metabolism were observed in experimental animals.

3. To determine antioxidant activities of plant extract in vitro studies.

4. Identification of bioactive compounds through GCMS, UV and FTIR Spectroscopic analysis.

5. Screening of protease producing bacteria from soil sample of mangrove forest region at Muthupett Thiruvarur District, Tamil Nadu, India.

6. Isolation, identification and molecular characterization of screened organisms.

7. Solid state fermentation of Brassica oleracea L.by selected organisms for enhancing Angiotensin I converting enzyme inhibitor activity.

8. To confirm antihypertensive compounds by insilico analysis.