2.0 INTRODUCTION

Diabetes mellitus, long back considered a disease of minor significance to world health, is now taking its place as one of the main threats to human health in the 21st century (Zimmet, 2000). The global prevalence of diabetes is set to rise from the current estimate of 150 million to 220 million in 2010 and 300 million in 2025 (Amos et al., 1997). Diabetes often leads to disability from the vascular complications of coronary artery disease, cerebrovascular disease, renal failure, blindness and limb amputations in addition to neurological complications ultimately resulting into premature death (Weidmann et al., 1993).

After the introduction of insulin treatment, life expectancy has increased and instead the problem with chronic complications evolved, cardiovascular disease and renal failure becoming the major causes of death among patients with diabetes (Paz-Guevara et al., 1975). Today, patients with diabetes still have an excess morbidity and mortality when compared with the general population, the major causes still being cardiovascular disease and renal failure (Rossing et al., 1996; Morrish et al., 2001; Orchard et al., 2001).

Unfortunately, chronic complications of diabetes show a rising trend among the patients living longer. Diabetes, formerly thought to be a problem of glucose metabolism, actually produces large number of micro and macro vascular complications affecting particularly cardiovascular system (Grundy et al., 1999). Patients with diabetes are characterized by an increased likelihood for developing congestive heart failure through increased coronary artery disease (CAD), hypertension, specific cardiomyopathy and endothelial dysfunction. Over the last three decades, a number of epidemiological, clinical and autopsy studies have proposed the presence of diabetic heart disease, as a distinct clinical entity (Zhi et al., 2004).

Cardiovascular diseases (CVDs) are the major causes of mortality in persons with diabetes and many factors including hypertension contribute to the high prevalence of CVDs. CVDs accounts for up to 80% of the deaths in person with type 2 diabetes (Haffner et al., 1998). There is an increasing recognition that diabetic patients suffer from an additional cardiac insult termed as ‘diabetic cardiomyopathy’. This entity was originally described in 1972 on the basis of observations in four diabetic patients who presented with heart failure without evidence of hypertension, CAD, valvular or congenital heart disease (Rubler et al., 1972). Other important risk factors for CVDs in diabetic patients include obesity, atherosclerosis, dyslipidemia, microalbuminuria (Dinnen and Gerstein, 1997), endothelial dysfunction, platelet hyperaggregability, coagulation abnormalities and diabetic cardiomyopathy (Goyal, 1999).

Cardiomyopathy associated with diabetes is a unique myopathic state that appears to be independent of macrovascular or microvascular disease and contributes significantly to CVD morbidity and mortality in diabetic patients, especially those with co-existent hypertension. Diabetic cardiomyopathy is characterized mainly by impaired diastolic function (Brown et al., 1996; Ren et al., 1996; Ren et al., 2000). Diabetic cardiomyopathy can act as the independent factor affecting the cardiac structure and function, and may also modulate prognosis of other complications such as ischemic heart disease (Nunoda et al., 1985; Savage et al., 1988; Lewinter, 1996).

Morphological changes in the diabetic cardiomyopathy include myocyte hypertrophy, necrosis, interstitial and perivascular fibrosis and capillary basement membrane thickening (Nunoda et al., 1985; Shehadeh and Regal, 1995; Lewinter, 1996). Functional abnormalities involve both the systolic and diastolic properties of myocardium, such as impaired relaxation, reduced compliance with elevated end diastolic pressure, cardiac hypertrophy and chamber
dilatation (Nunoda et al., 1985; Shehadeh and Regal, 1995; Lewinter, 1996). Further, up to 75% of CVD in diabetes may be attributable to hypertension, leading to recommendations for more aggressive treatment (i.e. reducing blood pressure to \(< 130/85 \text{ mm Hg}\)) in persons with coexistent diabetes and hypertension (Sowers et al., 2001). Hypertension is an extremely common co-morbid condition in diabetes affecting approximately 20-60% of patients with diabetes, depending on obesity, ethnicity and age (Arauz-Pacheco et al., 2002). Hypertension is approximately twice as frequent in patients with diabetes as compared with patients without the disease (Gress et al., 2000). Conversely, recent data suggest that hypertensives are more predisposed to the development of diabetes than normotensives (Sowers and Epstein, 1995).

In type 2 diabetes, hypertension is often present as a part of the metabolic syndrome of insulin resistance also including central obesity and dyslipidemia (Eriksson et al., 1992). Hypertension substantially increases the risk of both macrovascular and microvascular complications. Macrovascular complications include coronary artery disease, peripheral vascular disease, atherosclerosis, myocardial infarction, stroke, and gangrene whereas microvascular complications include small vessel diseases such as retinopathy, neuropathy and nephropathy. UK Prospective Diabetes Study Group (UKPDS) demonstrated that tight blood pressure control reduces the risk of fatal and non-fatal macro and microvascular diabetic complications (UKPDS 38, 1998; UKPDS 38, 1998a). According to UKPDS epidemiological study, each 10-mmHg reduction in mean systolic blood pressure was found to be associated with reductions in risk of 12% for any complications related to diabetes, 11% for myocardial infarction and 13% for microvascular complications. Hypertensive diabetes patients are also at an increased risk for diabetes-specific complications including retinopathy and nephropathy (Hasslacher et al., 1985; Hasslacher et al., 1985a; Knuiman et al., 1986).

Diabetes has become the number one cause of end-stage renal disease (ESRD) in the United States (US) and incidence of diabetes mellitus continue to grow both in the US and worldwide. Diabetic nephropathy may develop in 30 to 40% patients with diabetes mellitus (Andersen et al., 1983). However, recent studies suggest that the incidence in this group is declining (Bojestig et al., 1994). But still approximately 20 to 30% of all diabetics will develop evidence of nephropathy, although a higher percentage of type 1 progresses to ESRD. Approximately 45% of new patients entering dialysis in US are diabetics (Augustine and Vidt, 2004).

Diabetic nephropathy is the leading cause of ESRD in developed countries and leads to a heavy burden of dialysis and transplantation. The risk of premature death in patients with diabetic nephropathy is increased by the factor of 40-100, and other complications such as retinopathy and neuropathy cluster in these patients. Early studies suggested that the cumulative death rate of diabetic subjects with nephropathy was around 70% at 10 years (Borch-Johnsen et al., 1985). Although type 1 (insulin dependent) and type 2 (non-insulin dependent) diabetes are etiologically and epidemiologically different conditions affecting different segments of population, no major difference has been identified between the nephropathies seen in these conditions, either pathophysiologically or in terms of management.

Diabetic nephropathy is typically defined by either microalbuminuria, i.e. a urinary albumin excretion of greater than 300mg in a 24-hour collection or by abnormal renal function as represented by abnormality in serum creatinine, calculated creatinine clearance or glomerular filtration rate (GFR). The natural history of diabetic nephropathy is a process that progresses gradually over years. Early diabetes is heralded by glomerular hyperfiltration and an increase in GFR. This is thought to be related to increased cell growth and expansion in the kidney, possibly mediated by hyperglycemia itself. The common progression from
microalbuminuria to overt nephropathy has led many to consider microalbuminuria to define early or incipient nephropathy. Clinically diabetic nephropathy is characterized by a progressive increase in proteinuria, hypertension, decline in GFR and renal function, high risk of cardiovascular morbidity and mortality in patients with diabetes mellitus (Augustine and Vidt, 2004).

Early studies showed that systemic hypertension accelerates renal injury in diabetes (Mogensen et al., 1983) and rate of progression of renal disease is slow in normotensive patients as compared to type 1 diabetic patients (Jacobsen et al., 1999). An increase in the intraglomerular pressure has been suggested to promote progressive renal injury early in diabetic nephropathy (Hostetter et al., 1982). This hypothesis is supported by the clinical findings that a high GFR early in diabetes is a risk factor for later development of diabetic nephropathy as shown both in cross-sectional (Mogensen and Christensen, 1984) and longitudinal studies (Rudberg et al., 1992; Bangstad et al., 2002). The ability of the kidney to maintain a constant GFR over a range of renal perfusion pressure is called autoregulation and is present in condition known as overt diabetic nephropathy (Parving et al., 1984). There is an association between nephropathy, proliferative retinopathy and autonomic neuropathy (Spallone et al., 1994; Malik, 2000) and this could be due to the co-existence of two or more diabetic complications. On the other hand, autonomic neuropathy could possibly have an effect of its own to cause renal injury via higher blood pressure, renal vascular dilation, increased intraglomerular pressure and all of which could be caused by an impaired vascular autoregulation (Sundkvist and Lilja, 1993; Spallone et al., 1994). Studies have shown that microalbuminuria and autonomic neuropathy co-exist in patients with type 1 diabetes (Berglund et al., 1991; Clarke et al., 1999) and among patients without nephropathy, the prevalence of autonomic neuropathy is low (Meinhold et al., 2001). It may be critical link between hyperglycemia and chronic complications (Evans et al., 2003) or consequences of some other pathogenic mechanism, for example advanced glycosylation end products formation (Scivittaro et al., 2000).

Commonly practiced pharmacological treatments of diabetes mellitus include oral hypoglycemic agents and/or insulin injections (Lebovitz and Pasmantier, 1990). Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. Several such herbs have shown antidiabetic activity when assessed using present available experimental techniques (Coimbra et al., 1992; Kar et al., 1999; Jafri et al., 2000). Wide arrays of plant derived active principles representing numerous chemical compounds have demonstrated activity consistent with their possible use in the treatment of NIDDM (Ivorra et al., 1988; Marles and Farnsworth, 1995). For many years, people in Mexico have used plants to empirically treat diabetes. World ethanobotanical information about medicinal plants reports almost 800 plants used in control of diabetes mellitus. In traditional practice, medicinal plants are used to control diabetes mellitus in many countries. This has caused an increase in the number of experimental and clinical investigations directed towards the validation of the antidiabetic properties, which are empirically attributed to these remedies (Ivorra et al., 1989; Alarcon-Aguilar et al., 1998).

_**Lagerstroemia speciosa** L. (L. speciosa) (Lythracease), commonly known as Crepe Myrtle, grows widely in tropical countries including Philippines, India, Malaysia, China and Australia, is a popular folk medicine. Tea, prepared from the leaves of _L. speciosa_ has been used for the treatment of diabetes mellitus (Quisumbing, 1978; Matsuyama, 2000).

The antihyperglycemic effect of _L. speciosa_ has been demonstrated in animals and in in-vitro studies. When genetically diabetic mice (type II) were fed with a diet containing hot water extract from _L. speciosa_ for 5 weeks, their elevated blood glucose was significantly suppressed (Kakuda et al., 1996). In another study, when obese diabetic rats were fed with a
Introduction

diet containing the same extract for 12 weeks, their blood glucose levels were not suppressed, but HbA1C levels and body weights were lowered significantly (Suzuki et al., 1999).

In recent study, both hot water and methanol extracts of leaves of *L. speciosa* were shown to stimulate glucose uptake in 3T3-L1 cells in a manner similar to insulin and to inhibit adipose differentiation induced by insulin, isobutyl-methyl-xanthin and dexamethazone suggesting that plant may be useful for prevention and treatment of hyperglycemia and obesity in type II diabetics (Liu *et al*., 2001). Also reports say that tannic acid isolated from *L. speciosa* stimulates glucose transport and inhibits adipocyte differentiation in 3T3-L1 cells (Liu *et al*., 2005). The leaves of *L. speciosa* contain large amount of corosolic acid, which has been shown to possess antidiabetic properties (Murakami *et al*., 1993) and also contain significant amount of tannins (Hayashi *et al*., 2002).

Corosolic acid isolated from *L. speciosa* has been reported to decrease glucose and insulin levels and also reduce insulin resistance in KK-AY mice (Miura *et al*., 2006). In a bioassay-guided fractionation, employing glucose transport activity in Ehrlich ascites tumor cells, corosolic acid (2-hydroxyursolic acid) isolated from methanol extract of *L. speciosa* leaf, which showed a significant glucose transport-stimulating activity at a concentration of 1 uM (Murakami *et al*., 1993). Accordingly, oral formulations of an extract from the leaves of *L. speciosa* standardized to 1% corosolic acid (GlucosolTM) exerted marked decrease in blood sugar in type II diabetic individuals (Judy *et al*., 2003) and was also found to lower post challenge plasma glucose levels in vivo in humans (Fukushima *et al*., 2006).

In human diabetes, an elevated endogenous glucose production is mainly due to a high rate of gluconeogenesis which occurs in the liver (Gerich, 1988). So far there are no published reports of the effects of *L. speciosa* on hepatic glucose metabolism affecting enzymes like hexokinase and Phosphoenolpyruvate carboxykinase (PEPCK). Further, the effect of *L. speciosa* in diabetic complications is not yet explored. Though development of modern medicines have resulted in advent of modern pharmacotherapeutics including insulin, biguanides, sulfonylureas and thiazolidinediones, there is still a need to look for new drugs as no drug (except strict glycemic control with insulin) has been shown to modify course of diabetic complications.

In the light of above, objective of present investigation was to find out the role of *L. speciosa* on experimentally induced diabetes and diabetic complications.