CHAPTER I

INTRODUCTION AND LITERATURE REVIEWS

Diabetes mellitus is a disorder of the carbohydrate, fat and protein metabolism associated with a relative or absolute insufficiency of insulin secretion and with varying degrees of insulin resistance (Stefan,1991). It is characterized by fasting hyperglycemia or levels of plasma glucose above defined limits during a glucose tolerance test. Hyperglycemia is the major cause of diabetic vascular complications involving both microangiopathy and macroangiopathy such as heart diseases, cerebrovascular diseases, peripheral vascular diseases, retinopathy and nephropathy (Garcia et al.,1974; Anderson et al.,1983; Davis,1992; Stamler et al.,1993). The primary cause of morbidity and mortality in diabetic process, even after glycemia is controlled, is cardiovascular disease, particularly the manifestations of arteriosclerosis (Kannel and McGee,1979; Panzram and Zabel-Langhening,1981; Miccosi,Gallus and Pozza ,1987). A retrospective analysis of Cohort of 5210 diabetic patients revealed a mortality rate 1.3 times higher than in the general population of Warsaw (Krolewski et al.,1977). According to Framingham, incidence of coronary heart disease were 24.8 and 17.8 per 1000 in diabetic men and women in comparison with 14.9 and 6.9 per 1000 in nondiabetic men and women, respectively (Kannel and McGee,1979). The patients are often hospitalized because of angina pectoris, left ventricular failure, acute myocardial infarction or sudden death.

Cardiac dysfunction in diabetes mellitus has been observed in several studies. For example, Ahmed et al. (1975) found the abnormalities of the systolic time interval (STI) including a shorter left ventricular ejection time (LVET) and prolonged preejection period (PEP) in diabetes mellitus. An elevated PEP/LVET ratio is characteristic of heart failure. However the PEP/LVET ratio will be within the normal limit after 2-4 months of the therapy achieving a significant decrease in blood glucose concentration (Shapiro et al.,1981). In another study (Shapiro, Howat and Calter 1981), also found that isovolumetric relaxation is prolong. In BB diabetic rats show

depressed cardiac contractility and ventricular relaxation rates six weeks after the onset of diabetes when compared to BB nondiabetic littermates (Rodrigues and McNeill,1990).

Gotzche (1986) observed abnormally prolonged QT interval in diabetes subjects which may account for an increase frequency of ventricular arrhythmia resulting from the presence of abnormal myocardial depolarization. Additionally, the studies in an isolated working diabetic rat's hearts showed significantly depressed in peak left ventricular systolic pressure whereas left ventricular diastolic pressure was markedly increased. Authors also observed diminish heart rate while arterial pressure was elevated (Penpargkul et al.,1980; Ganguly,Thliveris and Mehta ,1990 ;Jermendy and Bachmann,1994).

By echocardiography, the end systolic volume in diabetes was greater than those of the normal subjects (Labadili and Goldstein,1983). But the left ventricular diastolic volume in 39 young IDDM patients were similar to those of the control subjects (Kimball et al.,1994). They also demonstrated that these patients had increases in left ventricular mass, performance, contractibility, systolic and diastolic pressure when compared with those of the healthy individuals. The authors indicated that the increase in left ventricular mass was due primarily to an increase in thickness. Similarly digitized M-mode echocardiography in 36 young IDDM women, the results revealed diabetic subjects with severe microvascular complication had thicker left ventricular wall (Airaksinen et al.,1984).

Morphologically, diabetic heart showed interstitial accumulation of periodic acid schiff (PAS) positive material, probably glucoprotein (Regan et al.,1974;Factor,Minase and Sonneblack,1980). A later study demonstrated an increase collagen concentration in heart of diabetic animals (Regan et al.,1981). These abnormalities may occur due to the small vessel disease. Crall and Roberts (1978) found intimal fibrous proliferation in the intramural coronary arteries of IDDM patients. PAS staining material of the media of some intramural arteries was also observed.

At necropsy, Waller, Pulumbo and Robert (1980) observed the severity of luminal narrowing of the left main coronary artery was greater in the patients with

diabetic mellitus than in the nondiabetic control. Besides, the right ,left anterior descending and left circumflex coronary arteries per patient were 75% narrowed in cross-section area by atherosclerotic plagues. The distally segments of the right and left circumflex coronary artery were narrower than the proximally segments whereas the left anterior descending coronary was similar both proximally and distally (Crall and Roberts,1978). The dominant component (91%) of the plaques consisted of fibrous tissue and nearly all of the remaining (9%) consisted of lipid deposits (Mautner, Lin and Roberts,1992). Scanning electron microscope revealed that the endothelial cells showed clear cell margin in nondiabetic rats. The cell surface was smooth and flat in most area. The central area appeared to be elevated in some cells. In diabetic BB rats, some areas of the aorta showed loss of cell margins, the cell surface was irregular and appeared swollen. Some cells had small holes covering part of the surface. These holes were seen both at the central and peripheral areas of the cell. Such abnormal-looking regions were interspersed with normal-looking areas (Meraji et al.,1987). An increased opening of the endothelial through junction region and the frequency of endothelial cell death were also reported in the aorta of diabetic animals (Dolgov et al.,1982; Lin et al.,1993). It would lead to an enhancement of transendothelial macromolecular transport such leaky junctions around the dead endothelial cells which in turn suggested that these changes may contribute to accelerated atherogenesis in diabetes. Generalized thickening of the capillary basement membrane was also found in diabetes (Williamson and Kilo,1976; Silver,Huckell and Lorber,1977; Fischer,Leskiw and Barner,1981) and capillary microaneurysms have been found in silicone rubber injected preparation of diabetic myocardium (Factor,Okum and Minase,1980).

Proteinuria and Dyslipidemia

Diabetic nephropathy is a serious complication accounting for 35% of the patients with insulin dependent diabetes mellitus (Mathiesen, 1993). The incipient diabetic nephropathy is defined as persistent levels of albumin excretion rate in the range of 20-200 mg/min or 30-300 mg/24hours or 0.46-4.6 mmol/24 hours (microalbuminuria) in at least 2 out 3 consecutive urine collection. The clinically

overt diabetic nephropathy occurs when albumin excretion rate persistently exceeds 200 mg/min (macroalbuminuria) or when total proteinuria rate exceed 0.5 g/24 hours (Jerums et al.,1994).

Recent studies have shown that an increase of urinary albumin excretion rate is an important predictor, not only of clinical diabetic nephropatthy, but also of cardiovascular even (Borch-Johnsen, Anderson and Deckert,1985; Jarett et al.,1984; Ah-kions and Fifth,1993). The number of cardiovascular deaths in patients who had proteinuria was 30 times higher than in those who did not develop proteinuria (Borch-Johnsen and Kreiner,1987). The cause of increased cardiovascular mortality in diabetic patients with increase urinary albumin excretion rat remains unknown. However, it has been shown previously that type I diabetic patients with nephropathy had elevated blood pressure (Jensen et al.,1987), increase fibrinogen concentration (Jones et al.,1989) and lipid abnormalities (Lahdenpera et al.,1994).

Usually, diabetic patients have an increased of frequency of dyslipidemia, which includes quantitative as well as qualitative abnormalities of lipoprotein and derangement in lipoprotein metabolism. However IDDM patients with microalbuminuria have higher prevalence of lipoprotein abnormalities (Jones et al.,1989). Hypertriglyceridemia with elevated cholesterol level are common in these patients. Other lipoprotein abnormalities include increase plasma levels of very low-density lipoprotein (VLDL), low-density lipoprotein (LDL) and apolipoprotein B, whereas levels of high-density lipoprotein (HDL) are typically reduced. The mechanism responsible of these lipoprotein abnormalities is probably related to the effect of insulin on adipose tissue lipoprotein lipase activity. Acute insulin deficiency initially causes an increase in free fatty acid mobilization from adipose tissue, resulting in increased secretion of VLDL-TG from the liver. Long-term insulin deficiency, the liver converts free fatty acids into ketone bodies, and VLDL-TG secretion diminishes. At the same time, lipoprotein lipase activity falls, resulting in impaired clearance of VLDL and chylomicrons from the plasma (Dunn,1992; Garg,1992). VLDL particles may be cholesterol enriched, and LDL particles may be rich in triglycerides (James and Pometta,1990). Treatment of diabetic with insulin rapidly corrects such metabolic abnormalities. In addition to, an increasing in platelet

aggregation, fibrinogen concentration and the decreasing of fibrinolytic activity have been also reported (Kwaan,1992).

These abnormalities may contribute to accelerate the atherosclerotic process causing cardiovascular complications in diabetic patients. Therefore, in addition to the control of hyperglycemia, the management of dyslipidemia, hypertension and proteinuria were essential and should be beneficial in diabetes.

Garlic

Garlic (*Allium sativum Linn.*), Liliaceae family, has been used world wide as a folk medicine to cure a wide range diseases. Garlic bulbs contain various kind of nutrients. The nutritional composition of 100 g. of garlic bulb are humidity 77%, protein 3.4%, fat 1.1%, carbohydrate (in the form of cellulose) 10.7%, vitamins 0.4%, minerals 0.8%, volatile oils and other 6.6%. The volatile oil is composed of sulfur-containing compounds (allyl disulfide, diallyl disulfide, diallyl trisulfide etc.). The bulbs contain about 0.24% by weight of an oderless, colorless sulfur-containing amino acid call alliin (S-allyl-L-cysteine sulfoxide, $C_6H_{11}NSO_3$) (Stoll and Seebeck,1951), which has no pharmacological activity. Evidently the cutting or crushing of garlic enables the enzyme, called allinase, to release which results in the conversion of alliin to 2-propenesulfenic acid. The end products are allicin (dially disulfide oxide, $C_2H_{10}S_2O$), pyruvic acid and ammonia (Block,1985; Block et al.,1986) as shown in Fig.1. Allicin is believed to be responsible for some of the pharmacologic activity of the plant (Garlic,1988). The quantitative differences of sulfur-containing compounds of garlic are primarily due to extraction procedure and the solvents that used for extraction. In freshly prepared chloroform extract of garlic (*Allium sativum*), allicin is the only major component (Sendl et al.,1992).

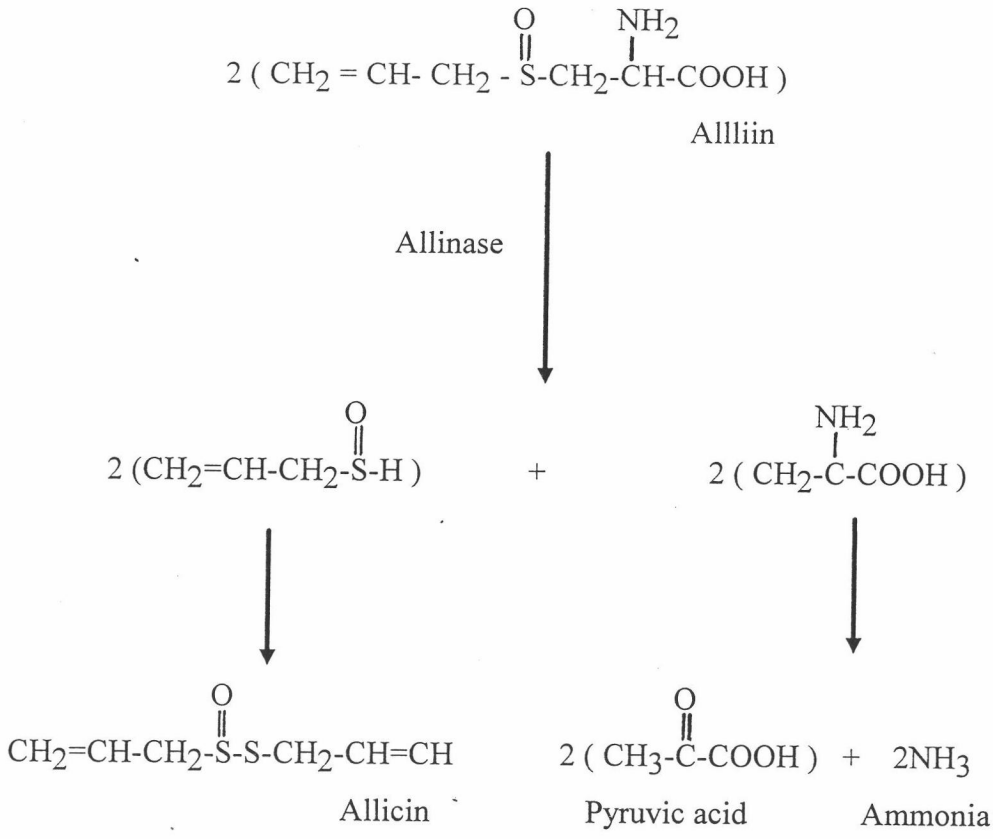

Fig.1 Chemical Reaction of Alliin-Allicin system

Pharmacological Effects of Garlic

Garlic has been used in herbal medicine for thousands of year. The hypoglycemic and hypotensive effect of garlic were reported. Jain, Vyas and Mahatma (1973) found that garlic had a slower and somewhat less potent effect than tolbutamide. Chang and Johnson (1980) demonstrated in rats fed a control diet containing 1% cholesterol and 46.8% sucrose or control diet plus 5% garlic. The result showed that garlic diet significantly reduced serum glucose as well as increased serum insulin and liver glycogen.

On cardiovascular actions of garlic, an average reduction of 12 to 30 mmHg systolic and 1 to 20 mmHg diastolic blood pressure can be induced by regular administration of garlic to patients with essential hypertension (Ernst, 1987; McMahon and Vargas, 1993). In anaesthetized dogs, garlic reduced arterial blood pressure (Pantoja et al., 1991) and decrease in heart rate.

The effect of garlic on plasma lipid was widely described. In animals studies, garlic oil showed decrease in serum and liver cholesterol (Kamanna and Chandrasekhara, 1984). In healthy subjects and patients with coronary artery diseases with elevated serum cholesterol, garlic decreased serum cholesterol, triglycerides and low-density lipoprotein while increasing high-density lipoprotein level. (Bordia, 1981). In addition, persons with cholesterol levels greater than 200 mg/dl, garlic decreased total serum cholesterol levels by about 9% (Warshasky, Kamer and Sivak, 1993) and low-density lipoprotein by 11% (Jain et al., 1993). These garlic effects have been always accompanied to the effects on inhibition of platelet aggregation and increasing plasma fibrinogen, fibrinolytic activity and coaggulability (Bordia et al., 1975; Jain, 1977; Gadkari and Joshi, 1991; Mansell and Reckless, 1991; Legnani et al., 1993).

Garlic caused not only indirect influence through an effect on atherosclerotic risk factors, but also direct action on the arterial wall (Orekhov et al., 1995). In induced atherosclerotic rabbits, the surface area involved by atherosclerotic lesions at aorta and coronary artery in garlic fed group was only half of that observed in the control group(Mand et al., 1985).

According to these attractive pharmacological effects of garlic, the present study has been conducted to access the effects of garlic on diabetic complications. Especially, on diabetic cardiovascular complications. Since there was no reports that has been directly performed yet.

Objectives

To study the effects of chloroform-garlic extract on serum insulin, serum lipid profiles and lipoprotein, cardiac functions, structure of coronary arteries, arterioles and capillaries, and protein in urine in streptozotocin induced diabetic rats.