CHAPTER I
INTRODUCTION AND LITERATURE REVIEWS

Diabetes Mellitus

Diabetes mellitus is a syndrome characterized by chronic hyperglycemia and disturbances of carbohydrate, fat and protein metabolism due either to an absolute deficiency of insulin secretion or a reduction in the biological effectiveness of insulin (or both) (Greenspan and Baxter, 1994; Kahn and Weir, 1994). Diabetes may be suspected or recognized clinically by the presence of characteristic symptoms such as excessive thirst, polyuria, pruritus, otherwise unexplained weight loss, or one or more of the many complications associated with or attributed to the disease (Kahn and Weir, 1994). Traditionally, diabetes has been classified according to the patient's age at onset of symptoms (juvenile-onset versus adult-onset) (Greespan and Baxter, 1994). In 1979, the National Diabetes Data Group recommended that diabetes mellitus can be divided into two major categories: insulin-dependent (type I) and non-insulin-dependent (type II) diabetes mellitus (Wilson and Foster, 1992; Greenspan and Baxter, 1994).

1. Type I: Insulin-dependent diabetes mellitus (IDDM)

IDDM was formerly known as juvenile-onset diabetes, ketosis-prone diabetes, immune-dependent diabetes and type I diabetes (Kahn and Weir, 1994). IDDM accounts for about 10% of diabetes, usually appeared in children, and is caused by selective destruction of insulin-secreting pancreatic beta-cell. There is general agreement that the key pathogenetic mechanism for beta-cells destruction of IDDM is autoimmune mediated. Contemporary research, particularly that using cellular and molecular methodologies, suggests that the pathogenesis of immunologically mediated diabetes depends on a wide range of factors (Rossini et al., 1993). From this and other causes result in deficiency in insulin secretion that lead to profound deficits in insulin action, plasma glycogen is elevated, and the pancreatic beta-

cells fail to respond to all known insulinogenic stimuli, In the absence of insulin, the three main target tissue of insulin (liver, muscle and fat) not only fail to appropiately take up absorbed nutrients but also continue to deliver glucose, amino acid, and fatty acid into the bloodstream from their respective storage depots, the more common manifestations of which are sever hyperglycemia and its immediately related symptoms (polyuria, thirst, polyphagia and weight loss). Insulin treatment could control hyperglycemia and symptoms. And this treatment also prevent the spontaneous occurrence of ketoacidosis (Greenspan and Baxter, 1994; Kahn and Weir, 1994).

2. Type II: Non-insulin-dependent (non-immune-dependent) diabetes mellitus (NIDDM)

NIDDM is common disease and constitutes 85% or more of all case of diabetes. NIDDM may present at any age. However, it could occur predominantly in adults and may occasionally have its onset in childhood. Most forms of NIDDM are associated with a positive family history of disease. NIDDM is also commonly associated with obesity.

NIDDM is the consequence of deficiency in insulin action due to abnormalities at the cell surface or within the beta-cells, a deficiency in insulin secretion less severe than that characterizing IDDM, or a combination of these processes. NIDDM can present with classical diabetic symptoms and sings such as thirst, polyuria, polyphagia, pruritus, and weight loss, but these usually appear only after a long asymptomatic peroid. Insulin treatment is not necessary to maintain life or prevent spontaneous ketosis in patient with NIDDM (Kahn and Weir, 1994).

Diabetic cardiovascular complications

Diabetes mellitus is associated with a specific cardiomyopathy. Moreover, diabetes is also associated with and increased risk of cardiovascular disorders, in particular, coronary heart disease (Miller, 1979). The hypothesis that diabetes mellitus provokes a specific cardiomyopathy is supported by numerous clinical, epidemiological and anatomopathological studies (Martinez, 1992). Cardiomyopathy as well as other cardiovascular diseases (e.g.

hypertension, atherosclerosis, and arteriosclerosis) account for nearly 80% of all diabetic deaths. The frequency of diabetic deaths due to cardiovascular diseases is two-to threefold higher than observed in the nondiabetic population. Coronary artery disease manifested by arterio-and atherosclerotic narrowing of the coronary vessels is though to be the major contributor to diabetic cardiomyopathy (Jackson et al., 1985). In clinical terms, diabetic cardiomyopathy is manifested both as an altered diastolic and/or systolic phase (Martinez, 1992). Therefore, onset of diabetes mellitus is associated with increased blood pressure, contractility, and left ventricular mass (Kimball, 1994). Several studies by using hearts of diabetic animals also shown diminished cardiac output, aortic output, and ventricular performance. The defect in cardiac performance with increased work load associated with acute insulin deficiency (Miller, 1979; Penpargkul, 1980). The studies on cardiac ultrastructure in diabetic animals found increase lipid deposition and progressive deterioration of the myocardial cell integrity. This deterioration was characterized by loss of contractile protein, vacuolization (swollen sarcoplasmic reticulum), myelin formations, myocytolysis, and contracture bands. These alterations paralleled the depression of cardiac function (Jackson et al., 1985). And, in diabetic patients also demonstrated that alterations of coronary circulation may contribute to the progressive deterioration of myocardium and to the pathogenesis of diabetic cardiomyopathy (Nitenberg et al., 1993). However, the pathogenesis of diabetic cardiamyopathy remains unclear. The alteration of the coronary circulation might be responsible (Martinez, 1992; Nitenberg et al., 1993).

Diabetes mellitus cause both macro- and micro-angiopathic abnormalities in the cardiovascular system. Diabetic macrovascular complications include both cardiac and peripheral vascular diseases (Becker, 1990). Atherosclerosis is a major macrovascular complication of diabetes, contributing to 60% of the mortality in diabetic patients (Hsueh and Anderson, 1992). The risk factors for atherosclerosis in diabetic patients are multiple, and

most are similar to those in the nondiabetic populations (Becker, 1990). A well-known hypothesis for the development of atherosclerosis is the "response-to-injury" theory. This hypothesis suggests that at least two pathways may lead to formation of intimal smooth muscle proliferative lesions. One pathway, demonstrated in hypercholesterolemia, involves monocyte and possibly platelet interactions, which may stimulate fibrous-plague formation by growth-factor release from the defferent cells. The second pathway involves direct stimulation of endothelium, which may release growth factors that can induce smooth-muscle migration and proliferation, and possibly autogenous growth-factor release by the stimulated smooth-muscle cells (Ross, 1986).

Diabetic microangiopathy accounts for much of the morbidity and mortality of diabetes. In insulin-dependent deabetes, about one-third of subjects develop severe microangiopathic complications with poor long-term prognosis. Major susceptibility factors for diabetic microangiopathy include duration of disease and probably quality of metabolic control (Barnett, 1991). Several studies in diabetic patients and animal model of diabetes, there are both histological and functional evidence that the vascular endothelium is abnormal (Arbogast et al., 1984; Meraji et al., 1987; Nitenberg et al., 1993). The abnormalities of endothelial function in diabetic microvessels include abnormal permeability (Patumraj et al., 1992) and release of endothelium-derived vasoactive substances (Taylor et al., 1992). Several studies by using chemically induced diabetes and spontaneously diabetic animal model have demonstrated impairment of endothelium-dependent relaxation (Oyama et al., 1986; Meraji et al., 1987; Durante et al., 1988; Kamata et al., 1989; Tesfamariam et al., 1990; Taylor et al., 1992). Some studies have documented an increase in sensitivity to norepinephrine in diabetic vessels (Abebe and Macleod, 1990; Taylor et al.., 1992: Taylor and Poston, 1994). From the many metabolic disturbance of diabetes, hyperglycemia is the most likely cause of endothelial dysfunction. Indeed, Tesfamariam and co-workers have reported the impairment of

endothelium-dependent relaxation in normal vessels following exposure to abnormally raised concentration of glucose (Tesfamariam et al., 1990; 1991; Tesfamariam and Cohen; 1992). The mechanism of development of microangiopathy is still incompletely understood. However, the studies in diabetic vessels have indicated that hyperglycemia lead to abnormal endothelium-dependent relaxation in these vessels through several mechanisms such as development of capillary basement membrane thickening (Barnett, 1991), activation of protein kinase C (Lee et al., 1989; Abebe and Macleod, 1990; Tesfamariam et al., 1990), together with increased production of vasoconstrictor prostanoids and the generation of oxygen-derived freee radicals (Tesfamariam et al., 1989; Barnett, 1991; Tesfamariam and Cohen, 1992; Shimizu et al., 1993) Moreover, hyperglycemia also activate polyol pathway and altered L-arginine metabolism (Taylor and Poston, 1994). These mechanisms can easily alter vascular cell functions and lead to the complications that have been described in diabetic patients and animals. It is not clear, however, which mechanism of injury plays the primary role in producing these alterations in cell function. Probably no one pathway is predominant and any predominance may not apply to all vascular tisues. Indeed, it is likely that more than one of these pathways is responsible for the vascular changes observed in diabetes (Kahn and Weir, 1994).

Hypertension is a common problem in diabetic patients. Hypertension in the diabetic patients is associated with an increased incidence of both microvacular and macrovascular complication. However, pathogenetic mechanisms are unclear (Drury, 1983). Several studies demonstrated that hypertension is a major factor that contributes to the development of vascular complication of diabetes mellitus. Indeed, the endothelium emerges as a key target organ of damage in diabetes, this damage is enhanced in the presence of hypertension. The mechanism of the pathophysiological effects of hypertension lies at the cellular level in the blood vessel wall. Hypertension affect blood vessels by altering shear stress, which is related

to vascular flow, blood viscosity, and other factors, and by altered production and activity of vasoactive substances. Moreover, hypertension result in endothelial cell modification include changes in shape, enhanced proliferation, increased intimal permeability to certain substances and intimal thickening with proteoglycan accumulation. Hypertension in the presence of diabetes markely accerelates the atheroscleretic process. Thus, hyperglycemia and hypertension are important factor that markedly alter the function and structure of endothelial cells. Moreover, other metabolic derangements that ocur in diabetes such as hypertriglyceridemia and enhanced oxidation, and glycosylation are likely to further damage endothelial cells (Hsuch and Anderson, 1992).

Garlic *(Allium sativum)*

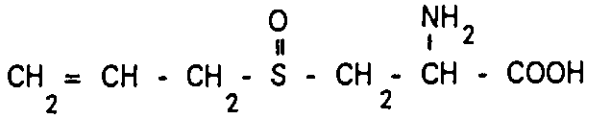
Garlic is number of the lily family which botanical name is ***Allium sativum*** ("allium" may derived from the Celtic word "all", which means pungent). It is among the oldest of all cultivated plants. Garlic is native to Asia and naturalized in South Europe and North America. The bulb has pungent aroma and teste. And it also is a classic flavouring agent in cooking (Block, 1985).

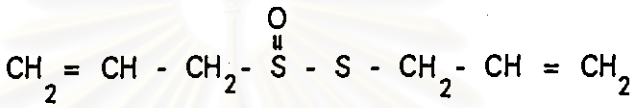
Chemical constituents

One of the earliest chemical studies was made in 1844 by the German chemist Theodor Wertheim. He suggested that garlic' appeal mainly to the presence of sulfur-containing components, called garlic oil. Furthermore, distillation of garlic oil yeilded some strong smelling volatile substances. Wertheim proposed the name ally (from Allium) for the hydrocarbon group in the oil, and schwefelallyl ("allylsulfur" in English) for the volatiles. "Allyl" is still used today. It refers to groups of structure $CH_2 = CHCH_2$, or in chemical shorthand C_3H_5.

Alliin or (+)-S-allyl-L-cysteine sulfoxide is the main sulfur-containing compound of garlic (Figure 1a). It could convert to diallyl disulfide oxide or diallyl thiosulfoxide which is called allicin by allinase or alliin lyase. The product of the reaction are pyruvate and ammonia (Figure 1b,c). Moreover, this reaction requires the participation of an edditional substance or cofactor called pyridoxal phosphate. Allicin is a chemically unstable, colorless liquid that accounts for the odor of garlic. Although allicin is responsible for the smell of garlic, a garlic bulb exhibit little or no odor untill it is cut or crushed (Block, 1985).

Pure allicin is an oily liquid. It is soluble in organic solvents such as alcohols, ketones, ethers, halogenated hydrocarbons, and aromatic hydrocarbons. (Jansen, 1987). At elevated temperature or especilly in fatty oil, allicin appears to be unstable. In these conditions, it could yeild several transformation products such as ajoenes, vinyldithiines and sulfides. And, these products could also play major role in discussion on garlic as a medicinal agent (Inberl, Winkler, and Knobloch, 1990).

a. 

b. 

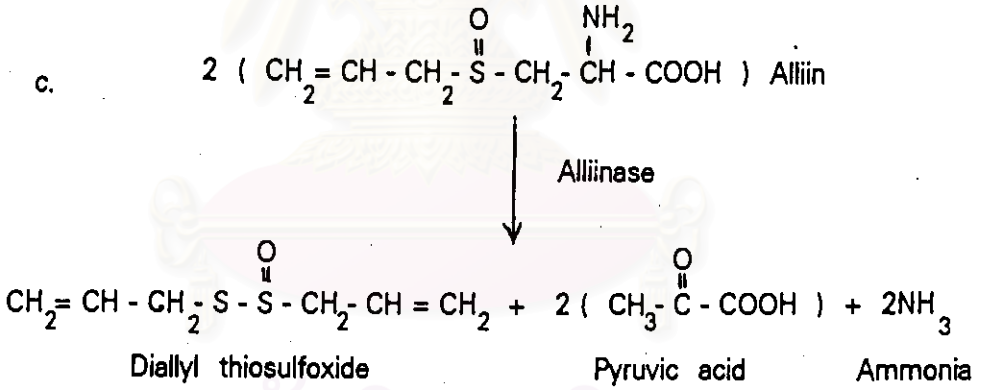
c. 

Fig. 1 a. Chemical structure of alliin.
b. Chemical structure of allicin.
c. Chemical reaction of alliin - allicin system.
(Block , 1985)

In order to obtain reliable quantitative determination of allicin, it has considered that solvents used for extraction may affect the activity of the allinase. Allinase is not inactivated by pentane, diethyl ether or chloroform whereas a methanol inhibits this enzyme (Jansen, 1987). It has been claimed that garlic extraction with chloroform gives pure allicin. The allicin extract obtained can be examined by thin-layer chromatography (TLC) at relative flow of 0.70-0.75 (Poolsanong, 1984).

Pharmacological effects

Garlic is widely used as a condiment and food. It has been also used in folk medicine in most culture for more than 5000 years. Further, chemical investigations on garlic have demonstrated that the active principle in garlic is allicin (Block, 1985; Iberl et al., 1990). In 1858 Louis Pasteur reported that garlic is antibacterial (Block, 1985). Later, several studies showed that it also is antimicrobial, antifungal and insecticidal (Fenwick and Hanley, 1985). Moreover, garlic can bring about plasma lipid normalization, enhancement of fibrinolytic activity, inhibition of platelet aggregation (Gadkari and Joshi, 1991; Kiesewetter et al., 1991), reduction in blood pressure (Sail and Ahman, 1981; Rashid and Khan, 1985; Kiesewetter et al., 1991) and glucose (Mathew and Augusti, 1973; Jain and Vyas, 1975; Chang and Johnson, 1980; Kiesewetter et al., 1991; Sheela and Augusti, 1992).

Functional and structural microcirculatory disorders were observed in more than 80% of patients suffering from diabetes mellitus or arterial hypertension. In the microcirculatory study, Kiesewetter et al. (1991) demonstrated that garlic could improve the microcirculation by increased erythrocyte velocity and decreased plasma viscosity in microvessels.

In the studies by using diabetic rats have demonstrated that garlic could increase heart rate, left ventricular contraction, aortic flow rate and coronary flow rate. Moreover, it could also increase high density lipoprotein-cholesterol, and decrease low density lipoprotein-

cholesterol and blood glucose (Anchalee Jatapai, 1994). The hypoglycemic effect of garlic was demonstrated by many workers and most of them attributed such effects to allicin type compounds (Sheela and Augusti, 1992). The possible mechanism of the hypoglycemic action of these garlic products was explained as that they may be potentiating the insulin effect by enhance transport of blood glucose to peripheral tissue (Jain and Vyas, 1975), or by increasing either the pancreatic secretion of insulin from the beta-cells of the islets or its release from bound insulin (Chang and Johnson, 1980).

Garlic extract could produce a significant fall in systolic, diastolic and mean blood pressure. It also decrease in total peripheral resistance (Ernst, 1987). Sail and Ahmad (1982) have demonstrated that the mechanism of hypotensive effect was earlier thought to be due to endogenous release of various hypotensive substances in the body. Later, Rashid and Khan (1985) postulated that hypotensive effect of the garlic extract may be either due to its prostaglandin-like content or to release of prostaglandins that was blocked by antagonist of prostaglandin, flufenamic acid. However, from the studies by using isolated guinea pig aorta and rabbit hearts have shown that hypotensive action of garlic juice may be due, at least in part, to a direct relaxant effect on arterial smooth muscle (Aquel, Gharaigah, and Salhab, 1991). Recently, the mechanism of the relaxant effect on arterial smooth muscle has been investigated by Ozturk et al., (1994). His finding obtained strongly suggested that the vasorelaxant effect of garlic is important in its hypotensive activity and mediated by production of endothelium and/or muscle-derived relaxing factors.

From the review literatures mentioned above, it was indicated that there is no experimental research studied of the effects of garlic extract on coronary arteriolar responses to vasoactive agents. Therefore, the major purpose of this present study is to asscess this effect matter directly by using streptozotocin-induced diabetic rats as an animal model.

The objectives of this study are :

1. to study coronary arteriolar responses to vasoactive agents in STZ-rats.

2. to study the effects of garlic extract on coronary arteriolar responses to vasoactive agents in STZ-rats.