CHAPTER V

DISCUSSION AND CONCLUSION

The present study primarily investigated subchronic effects of *Pueraria mirifica* on isolated aorta in high cholesterol-fed rats and ovariectomized rabbits. This would partly give a preliminary information of *P. mirifica* potential to improve endothelial function. Subchronic effects of *P. mirifica* on blood clinical biochemistry parameter were also investigated.

Part 1 in the present study included the high cholesterol-fed rats. As the cholesterol-fed animals model previously has been shown abnormal endothelium-dependent vascular relaxation It has been suggested by the other study that cholesterol feeding may alter endothelial cell (Minor *et al.*, 1990).

Part 2 was studied in ovariectomized rabbits which has been previously shown to be an appropriate model for studies of estrogen and atherosclerosis (Bjarnason *et al.*, 1997). Ovariectomy also produces a reduction of constitutive NOS activity which induces endothelial dysfunction (Catania *et al.*, 2002).

1. Effects of *Pueraria mirifica* in high cholesterol-fed rats.

1.1 Effects on body weight gains, blood biochemistry and serum lipid parameters.

Body weight gain of rats given *P. mirifica* were lower than in control and cholesterol groups. These were consistent to the results reported by Civapat and coworkers (2000). These effects were possibly due to the effects of some phytoestrogens containing in *P. mirifica*, such as miroestrol which shown to cause nausea and vomiting in human (Cain, 1960), genistein and daidzein which found to suppress food intake and body weight gain in rats (Toda *et al.*, 1999).

In this study, treatments with *P. mirifica* in high cholesterol-fed rats significantly reduced serum total cholesterol, triglyceride and LDL-cholesterol. This is in accordance with the experiments in male and female rats which shown that *P.mirifica* decreased serum cholesterol without any serious toxic effect (ทรงพล ชีวพัฒน์ และคณะ, 2543). *P.mirifica* composes of natural compounds of phytoestrogen like miroestrol, deoxymiroestrol, daidzein, genistein, kwahurin etc. The phytoestrogens in *P. mirifica* is shown to significantly reduce plasma total cholesterol, triglyceride and LDL-cholesterol in cholesterol-fed rabbit (Ratanachamnong *et al.*, 2000). In addition, Anderson *et al.*, (1995) demonstrated that genistein and daidzein significantly decreased serum concentrations of total cholesterol, triglyceride and LDL-cholesterol. It can be concluded that dietary treatment with *P. mirifica* would decreased serum lipid parameter.

The possible mechanism of the cholesterol reduction is an activation of the LDL receptor activity, increasing LDL catabolism and increasing the excretion of bile acids (Sanjay *et al.*,2000). Especially in the case of the cellular LDL receptor, excess cholesterol in the circulation will reduce expression and also reduce the capacity of cell to catalyze LDL, eventually leads to lipoprotein accumulation in the

arteries (Sirtori, 2002). In the present study of isoflavones, cellular LDL receptor, depressed by hypercholesterolemia, returned to normal function, thus allowed cell to more efficiently disposed of cholesterol (Sanjay *et al.*, 2000 and Sirtori, 2002).

1.2 Effect of vascular function in high cholesterol-fed rats.

Endothelial cells play numerous physiological roles including a permeability barrier through which there is exchange and active transport of substances into the arterial wall and maintenance of vascular tone by release of small molecules such as NO. Change in one or more of these properties may represent the earliest manifestation of endothelial dysfunction (Ross, 1993).

Endothelial dysfunction may therefore be a stepwise progression where damage to the endothelial membrane receptor is an initial event which is later followed by more severe and nonspecific perturbation of the synthesis and release of NO (Ross, 1993). Nitric oxide acts as a vasodilator when produces by constitutive NOS, which is composed of eNOS and nNOS in endothelial cells (Sun *et al.*, 2002). Impaired responses of arteries to endothelium-dependent vasodilation have been previously shown in cholesterol-fed rabbits (Habib *et al.*, 1986 and Keaney *et al.*, 1994).

In this study, phytoestrogens in *P. mirifica* restored the impaired relaxation response to acetylcholine in aortas isolated from high cholesterol fed rats. The contractile response to noradrenaline and the relaxation response to sodium nitroprusside did no differ from control.

The data presented the preservation of endothelium-dependent relaxation function in cholesterol-fed rats with *P. mirifica*. With regard to the responses observed in the cholesterol *P. mirifica* group, the cholesterol lowering effects of *P. mirifica* could favorable affect endothelial relaxation function. There are also experimental studies demonstrating the protection against atherosclerotic development by genistein and daidzein in male cynomolgus monkeys (Anthony *et al.*, 1997). Animals fed phytoestrogens had significant lower total cholesterol and LDL plus VLDL cholesterol concentrations (Anthony *et al.*, 1997). Clarkson *et al.*, (1998), reported that genistein and diadzein showed lowering effects on LDL and also inhibit the progression of atherosclerosis in coronary, iliac, common and internal carotid arteries in human. Puerain, an isoflavones from plants of the genus *Pueraria*, has been widely used in the treatment of angina and other ischemic diseases (Sun *et al.*, 2002).

2. Effects of *Pueraria mirifica* in ovariectomized rabbits.

2.1 Effects on body weight gains and blood biochemistry and serum lipid parameters.

No significant differences was demonstrated between the groups in body weight gain, blood biochemistry and serum lipid parameters in the same period. However, there was a tendency toward a lower total cholesterol and LDL-cholesterol content in the OVX+ *P. mirifica* group.

Similar results were reported in the previous study with the treatment of 17β-estradiol in ovariectomized rabbits. These results showed that serum level of cholesterol and HDL-cholesterol did not differ between untreated and estrogen treated animals but serum triglyceride were less in the estrogen-treated group than in the untreated group (Virginia *et al.*, 1990). But Clarkson *et al.*, (2001) reported that phytoestrogens effect on plasma lipid shown no significant difference between group in ovariectomized cynomolgus monkeys. This discrepancy may be due to the differences in approximate dose, the species, the duration of treatment and the content of cholesterol in the diets.

2.2 Effect of vascular function in ovariectomized rabbits.

The main findings of our study are that treatment of ovariectomized rabbits with 17β-estradiol or *P. mirifica* enhances cholinergic response, endothelium-dependent relaxation, of the aorta. These effects occured without any change in the sensitivity of smooth muscle cells to either NO donor or an adrenergic receptor agonist.

Mechanisms underlying reduced NO activity in ovariectomized induced endothelial dysfunction are yet unclear. It could theoretically be due to a decreased synthesis of NO which may be due to a decreased ability of NO production in endothelial cell (Moncada and Higgs, 1993).

In the present study, 17β-estradiol reversed the depression in the endothelium-dependent response to acetylcholine in ovariectomized rabbit aorta. The salutary effects of 17β-estradiol on endothelial function in ovariectomized rats were previously reported (Rahimian *et al.*, 1997). Collins *et al.*, (1994) reported that coronary artery from rabbits treated with 17β-estradiol showed an enhance endothelium-dependent relaxation to acetylcholine. This study the effect of 17β-estradiol in ovariectomized

rats by Binko *et al.*(1998). It has been proposed that an increased endothelium-dependent relaxation to Ach maybe result from an induced iNOS. In addition, the study of Holm *et al.* (1997) indicated that 17β-estradiol upregulates the transcription of constitutive NOS in the endothelial cell and has the direct antiatheroginic effects on arterial wall by mediate through its effect on endothelial NO in rabbits.

Acetylcholine-induced endothelium –dependent relaxation were reduced at $1x10^{-5}$ to $1x10^{-4}$ M acetylcholine concentrations. This is due to the evoked contraction by high concentration of Ach .The endothelium-dependent contraction observed in ovariectomized rabbits aorta following acetylcholine stimulation is potentiated by inhibitors of NO production such as EDCF and oxyhemoglobin (Bonlanger and Vanhoutte, 1994)

In the present study, phytoestrogens in *P. mirifica* restored the impaired endothelium-depentdent relaxation response to Ach in ovariectomized rabbits. Another phytoestrogens such as genistein and daidzein are also reported to produce also an improvement of endothelial dysfunction induced by ovariectomized rats. (Catania *et al.*, 2002).

Mechanisms of phytoestrogens in *P. mirifica* restored endothelial dysfunction are yet unclear. Evidence has accumulated to suggest that oxLDL is a key component in endothelial injury. OxLDL may directly injure the endothelium and play an initial role in the increased adherence and migration of monocytes and T lymphocytes into the subendothelial space. It can induce of atherosclerosis (Ross, 1993).

A possible mechanism of antioxidant mediated preservation of endothelial function is a decreased oxidative modification of LDL. It was found that restoration of endothelial function was associated with increased resistance to LDL oxidation (Tikkanen and Adlercreutz, 2000). Isoflavones had beneficial effects on plasma lipid concentration such as reduction in LDL cholesterol and increase in HDL-cholesterol in premenopausal cynomolgus monkeys and provide protection against oxidative modification of LDL. (Tikkanen *et al.*, 1998 and Clarkson *et al.*, 2001). Phytoestrogens may also prevent the oxidation of lipoprotein particles. The phytoestrogens in *P. mirifica* reduced plasma total cholesterol, triglyceride and LDL-cholesterol significantly in hypercholesterolemic rabbit and increase the oxidation resistance of LDL (Ratanachamnong *et al.*, 2000).

The changes in vascular function were also in accordant with the structural changes of vascular studies. Endothelial injury in the ovariectomized group is evidenced by accumulation of WBC in the luminal surface of the blood vessels. Together with the lobular appearance of the vessel surface may indicate the accumulation of lipid-filled macrophages or foam cells in the subendothelial layer. The previous study shows that the abnormal endothelium-dependent vascular relaxation is observed in atherosclerotic vessels (Harrison *et al.*, 1984). In addition, Kenaney *et al.*, (1994) have demonstrated that the abnormal endothelial cells with underlying foam cell formation are found in the cholesterol-fed rabbit. Whereas, reinstallment of vascular function controlled through the endothelium are restored by estrogen or *P. mirifica.*

In conclusion, our study show that *P. mirifica* improved endothelium-dependent relaxation in high cholesterol-fed rats. It maybe due in part to its cholesterol lowering effect. Whereas, the antioxidative effect may in part be responsible to the restored vascular function in ovariectomized rabbits. The results from the present study supports the benefit of traditional use of *P. mirifica* in Thai folk medicine. If may be used as an alternative to traditional hormone replacement therapy for menopausal women. However, future studies are needed to determine the main contents of phytoestrogens in *P. mirifica* and their estrogenic activity. As well as, to clarify the mechanisms of *P. mirifica* effects on vascular function, as well as to determine whether its impact on the endothelial cell may contribute to a restoration of vascular function in menopausal women.