CHAPTER I
INTRODUCTION

1. Rationale and Background

Diabetes mellitus (DM), a most common non-infectious disease, is regarded as the most common serious chronic metabolic disorder characterized by hyperglycemia. It can induce various functional and structural disorders both in the central and peripheral nervous systems (Biessels et al., 1994). In addition, it also induces a long-term complications affecting the eyes, kidneys, nerves, heart and blood vessels (Gispen and Biessels, 2000 and McCall, 1992). It has been known that DM is a chronic disease that cannot be completely cured, therefore, the continually treatment is required in order to compensate the defect functions and for both as the treatment and limit the development of its devastating complications.

Recently, it has been reported that approximate 6.2% or 18.2 million people of the US population in 2002 were suffered from diabetes mellitus and the annual cost of healthcare related to this condition per person in that year was about $13,243. This amount was about 6 times higher than those of people without diabetes ($2,560) (Votey**,** 2005). Similar situation was also found in Thailand, it has been reported that approximate 4-7% of the population at age of 30-60 years suffered from diabetes and this prevalence was increased to 10-15 % of the population in elderly age over 60 years (Thaihealth, 2005). Therefore, diabetes is regarded as one important issue that should be considered.

It has been reported that the common important complications in diabetes are microvascular and macrovascular diseases (Votey, 2005), poor wound healing and temporary or permanent nerve damage. Moreover, it was also found that diabetes interfered with the peripheral nerve regeneration (Kennedy and Zochodne, 2000).

Previous studies had demonstrated that diabetic patients are highly susceptible to acute nerve compression or entrapment injuries (Chammas et al., 1995 and Stevens et al., 1993). Compression may result in demyelination and axonal degeneration (Dyck et al., 1990), and unfortunately there is a less favorable recovery in diabetics

after decompression surgery (Ward, 1997 and Haupt et al., 1993). Therefore, the nerve injury in diabetic condition is regarded as one of the challenges issue for this decade.

Since the nerve injury in diabetic condition is one of an important issues that should be considered, the endeavor to search for strategies to improve functional recovery of nerve injury in diabetic condition should be focused. In the past decade many strategies to improve the functional recovery of nerve after injury have been developed including the application of numerous substances to accelerate functional recovery of nerve. Previous studies demonstrated that the ability to regenerate nerve was reported to be associated with level of antioxidant such as vitamin E (Enrione et al., 1999). Recently, numerous medicinal plants possessing potent antioxidant were reported to improve the functional performance and promote neurite outgrowth (Li and Ohizumi, 2003). It was proposed that these plants could increase growth factor level, an important factor for promoting the recovery process after nerve injury (Li and Ohizumi, 2003).

In addition to peripheral nerve injury in diabetic condition, diabetic wound are also regarded as the challenge of this decade. The wound healing outcome in diabetic condition is still not in satisfaction level. It has been reported that wound healing process in diabetic condition is delayed by various factors including infection, collagen synthesis impairment and poor blood supply. Previous study showed that diabetes mellitus could increase oxidative stress which in turn contributed the important role on the pathophysiology of numerous complications including wound healing impairment. It was found that the oxidative stress imbalance could produce lipid peroxidation which in turn interfere the biosynthesis and metabolism of keratinocyte, endothelial cells, fibroblast and collagen (Yavuz et al., 2005)resulting in wound healing impairment.

At present, the effectiveness of therapeutic strategy of both peripheral neuropathy and wound healing process in diabetic condition is still very limited. Thus, the novel therapeutic strategies against these complications are still essential.

Recent studies demonstrated that substances possessing antioxidant activity could enhance functional recovery of nerve (Tawata et al., 2004) and wound healing (Kwon et al., 2008). Since, Thailand is abounding for abundant plants and vegetable

reputed for antioxidant activity, the application of the plant products for therapeutic purpose has gained much concentration. Based on the crucial roles of oxidative stress on the pathophysiology of peripheral nerve injury and wound healing retardation and the benefit of antioxidant on the mentioned condition, the development of novel strategy to facilitate the functional recovery of nerve and wound healing from natural product have been focused.

Among various natural products claimed for medicinal purposes which available in the market nowadays, *Quercetin* and Tomato are very much in used for various purposes. Both substances have been reported to possess antioxidant effect (Mook-Jung et al., 1999 and Heo and Lee, 2004). Moreover, they also exhibited the protective effect against oxidative stress injury (Mook-Jung et al., 1999 and Heo and Lee, 2004).

Taken all together, it was hypothesized that these substances should have the potential to facilitate the functional recovery of nerve and wound healing in the diabetic conditions. No scientific evidence on this issue was available. Thus, the present study was carried out to evaluate the potential of the mentioned substances to facilitate the functional recovery of nerve and wound healing process in diabetic condition. However, the oral administration is usually subjected to the first pass effect, the effective dose is often high and easily to produce adverse effect. Recent studies demonstrated that the transdermal application especially the transdermal application which used the nanofiber as vehicle system for drug delivery was very much effective at lower drug concentration (Deng-Guang et al., 2009). Therefore, the development of nanofiber-loaded natural products as the novel strategy to protect against peripheral neuropathy and diabetic wound was considered.

2. Objectives of the Study

2.1 General experiment aims

This experiment was designed to develop herb-based health product to facilitate the recovery of nerve functional and wound healing in diabetes mellitus.

2.2 Specific objectives

2.2.1 To screen the potential of quercetin and tomato to facilitate nerve function after injury and to enhance wound healing process.

2.2.2 To develop and to test the property of natural products (*Quercetin* or Tomato) loaded zein based nanofiber mats.

2.2.3 To determine the biological activities of the developed natural products (quercetin or tomato) loaded zein based nanofiber mats.

2.2.4 To test the efficacy of the natural products (quercetin or tomato) loaded nanofiber mat to enhance nerve functional recovery after injury in diabetic condition.

2.2.5 To develop and to test the efficacy of the natural products (quercetin or tomato) loaded nanofiber mat to enhance diabetic wound healing.

2.2.6 To determine the possible underlying mechanism of the natural products (quercetin or tomato) loaded nanofiber mats by focusing on the alterations of oxidative stress damage markers including the level of malondialdehyde (MDA) and the alteration of scavenging enzymes activities such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in the lesion nerve and in wound of diabetic rats.

2.2.7 To determine the axon density in the lesion nerve of diabetic rats.

2.2.8 To determine the collagen thickness in the wound of diabetic rats.

2.2.9 To determine the alteration of pERK ½ in the lesion nerve of diabetic rats

2.2.10 To determine subchronic toxicity of the developed natural products (quercetin or tomato) loaded zein based nanofiber mats.

3. Scope and Limitation of the Study

3.1 All animals used in this experiment were male only and the ages were approximately at 8 weeks old (young adult).

3.2 All animals used in this experiment were induced diabetes mellitus by the injection of glucosamine–nitrosourea compound streptozotocin (STZ) at dose of 50 mg/kg via intraperitoneal route.

3.3 All injections were performed at the same period to avoid the influence of circadian rhythm.

3.4 All stressors were avoided to prevent the influence of stress.

3.5 In all experiments, control and experimental groups were performed in parallel at the same period to avoid the effect of seasonal changes.

3.6 The traumatic lesion of nerve in this experiment was performed by using crush injury at right sciatic nerve.

3.7 The wound healing process was studied in model of excision wound.

4. Hypothesis

4.1 If quercetin or tomato have the potential to be served as resources for the development of the novel health products protecting against diabetic complications such as traumatic peripheral nerve injury and wound in diabetic condition, they should show the optimal benefits on the alteration of biological activities associated with the pathophysiology of the as traumatic peripheral nerve injury and wound healing process such as antioxidant and aldose suppression activities.

4.2 If quercetin or tomato loaded nanofiber mats can be used as the health product to facilitate nerve recovery and wound healing process, quercetin and tomato should be successfully loaded in to zein based nanofiber mats and can release from the nanofiber mats. In addition, the quercetin or tomato loaded nanofiber mats should still exert the antioxidant and aldose suppression activities at an acceptance level after subjecting to the electrospining process.

4.3 If the quercetin or tomato loaded zein based nanofiber mats have the potential to facilitate the functional recovery of nerve after traumatic lesion in diabetic rats, the diabetic rats which subjected to crush injury at right sciatic nerve and received quercetin or tomato loaded zein based nanofiber mats should show better functions of sciatic nerve than the diabetic rats plus right sciatic nerve lesion which received zein based nanofiber.

4.4 If quercetin or tomato loaded zein based nanofiber mats exert the beneficial effect via the decreased oxidative status, the lesion nerve of diabetic rats which subjected to crush injury at right sciatic nerve and received quercetin or tomato loaded zein based nanofiber mats should show lower oxidative stress status than that of diabetic rats which subjected to crush injury at right sciatic nerve and received zein based nanofiber.

4.5 If quercetin or tomato loaded zein based nanofiber mats exert the beneficial effect via the decreased polyol pathway function, the lesion nerve of diabetic rats which subjected to crush injury at right sciatic nerve and received quercetin or tomato loaded zein based nanofiber mats should show lower activity of aldose reductase than that of diabetic rats which subjected to crush injury at right sciatic nerve and received zein based nanofiber.

4.6 If quercetin or tomato loaded zein based nanofiber mats exert the beneficial effect via the increased pERK1/2, the lesion nerve of diabetic rats which subjected to crush injury at right sciatic nerve and received quercetin or tomato loaded zein based nanofiber mats should show higher level of pERK12 than that of diabetic rats which subjected to crush injury at right sciatic nerve and received zein based nanofiber.

4.7 If the quercetin or tomato loaded zein based nanofiber mats have the potential to facilitate the wound healing process in diabetic condition, the diabetic rats which subjected to crush injury at right sciatic nerve and received quercetin or tomato loaded zein based nanofiber mats should show better healing of wound than the diabetic rats which subjected to crush injury at right sciatic nerve and received zein based nanofiber

4.8 If quercetin or tomato loaded zein based nanofiber mats improve wound healing process via the decreased oxidative stress status at the wound, the wound of the diabetic rats which subjected to crush injury at right sciatic nerve and received quercetin or tomato loaded zein based nanofiber mats should show lower oxidative stress status than that of the diabetic rats which subjected to crush injury at right sciatic nerve and received zein based nanofiber

4.9 If quercetin or tomato loaded zein based nanofiber mats improve wound healing process via the increased collagen at the wound, the wound of the diabetic rats which subjected to crush injury at right sciatic nerve and received quercetin or tomato loaded zein based nanofiber mats should show higher level of collagen type I than that of the diabetic rats which subjected to crush injury at right sciatic nerve and received zein based nanofiber.

4.10 If quercetin or tomato loaded zein based nanofiber mats are safe for using as novel health products, the diabetic rats which received quercetin or tomato loaded zein

based nanofiber mats should show no significant difference in hematological changes or clinical chemistry changes than that of the diabetic rats which subjected to crush injury at right sciatic nerve and received zein based nanofiber. If the significant change was observed, the value of the parameters should be in the normal range.

5. Anticipated Outcomes

5.1 To provide the novel strategy to facilitate functional recovery and wound healing in diabetic condition.

5.2 To produce strategy for creating value-added of the natural product.

5.3 To increase the competition potential of health product developed from natural product in the market.

5.4 To decrease the disability induced by diabetic complication.