Chapter 1

Introduction

1.1 Background

Diabetes is a chronic metabolic disorder where the body experiences impairment in utilizing sugar, resulting in a high and unusual blood sugar level. Diabetes may happen due to the inability of the body to secrete insulin corresponding to the glucose level, peripheral insulin resistance (failure of the target tissue to dispose of glucose in response to insulin), or both (Goyal & Jialal, 2019). According to Khan *et al.* (2019), diabetes is one of the ninth leading diseases that causes mortality, with 1 million deaths per year from diabetes alone. Along with the disease itself, such complications may arise, one of them causing bruises that do not heal and can lead to a loss of blood supply to the tissue, resulting in gangrene and amputation (Deshpande, Harris-Hayes & Schootman, 2008). A study by Larsson, *et al.* (1998) shows that patients with amputation had a higher mortality rate, along with the incidence of diabetes patients suffering from complications ranging from 9.1 to 26.1 million (Oliver & Mutluoglu, 2022). Therefore, it is necessary to produce a better treatment to treat peripheral vascular diseases or foot ulcers caused by diabetes.

Wound dressing is believed to play a crucial role to manage diabetic foot ulcers as it can enhance the wound healing process while also protecting the open wound from the outside environment, preventing them from colliding with microorganisms that might cause secondary infections (Kavitha, 2014). One of the most advanced treatments for diabetic foot ulcers is bio-cellulose (BC), which is a cellulose derived from bacteria such as *Komagateibacter.* BC is able to provide protection against foreign materials while also scientifically proven to be biocompatible, biodegradable, and non-toxic compared to other dressing materials, therefore would be suitable to manage skin injury caused by DFU (Naomi & Fauzi, 2020). The incorporation or usage in combination with active ingredients with

an activity that could assist wound healing such as antimicrobial, tissue regeneration, and anti-inflammation would be beneficial. This mechanism might be able to be enhanced with the help of natural, anti-inflammatory agents such as green tamanu oil (Krist, 2020).

Green tamanu oil (GT) originates from the oil of the Tamanu tree (*Calophyllum inophyllum L.*) and it is known to treat various diseases in the medical field, especially skin diseases. One of the natural neoflavonoids that GT consists of is calophyllolide (CP) which is an anticoagulant and anti-inflammatory while also having healing properties (Krist, 2020). These various properties proposed GT to be applied to DFU patients to support the wound healing process.

The healing process of diabetic patients mainly experiences a disruption in the angiogenesis process, prolonged inflammatory conditions, and an imbalance in the regulation of the ECM (Spampinato, S. F. *et al.,* 2020). This is due to the elevated numbers of neutrophils and macrophages in diabetic patients, where they release inflammatory cytokines such as TNFα and IL-1β, which they usually only formed during acute inflammation. However, in diabetic patients, these cytokines numbers remained high even after the acute inflammatory repair phase, which causes prolonged inflammation in the wounded area. Moreover, some growth factors such as IGF-1 which is responsible for wound re-epithelialization, and TGFβ, responsible for recruiting immune cells, keratinocytes, fibroblasts, and structuring ECM also experienced a decrease in wound tissue of diabetic animals and humans (Spampinato, S. F. *et al.*, 2020). Some studies have shown that BC and GT are able to exhibit anti-inflammatory properties to enhance the wound-healing process.

The study of the efficacy of BC with GT will be conducted in two animal models which are mice and rabbits. Mice models have been widely used in research to study human diseases as it shares a homology genetically with humans (Kottaisamy *et al.,* 2021). Other than mice, rabbits are also going to be used in this experiment as they are found to be convenient and have a similar thickness to

human skin (Jung & Maibach, 2014). This study used two different types of animal models to further investigate and confirm the wound healing assessment of BC and GT on a more advanced diabetic animal model.

Diabetes can be chemically induced in these animal models by injection of Streptozotocin (STZ). Streptozotocin works by damaging the pancreatic β cells, therefore causing hypoinsulinemia and hyperglycemia. Streptozotocin is used as the diabetic induced chemical as it has been found to be safe and specific to cause necrosis to the pancreatic β cells (Damasceno *et al.*, 2014). The dose for each animal model can be adjusted according to the animal's physiology. Previous research has shown interest to determine wound healing capabilities, however, they were mainly focused on normal animal models which do not reflect diabetic wound healing conditions. Therefore, this study would like to focus on the wound-healing effects of BC with GT on diabetic animal models, while also measuring antimicrobial properties, the effect of treatment between two animal models, and histology studies.

1.2 Objective

The aim of this study is to investigate wound healing efficacy by wound size reduction measurement, histology observation of skin tissue, and antibacterial properties of the wound dressing treatment on diabetic-induced mice and rabbits.

1.3 Hypothesis

The following are this study's hypothesis:

1. BC dressing with GT would improve the wound size reduction and tissue regeneration through histology compared to gauze (negative control) on diabetic-induced animal models
2. The microbial count of BC dressing with GT would be the fewest when compared to control and/or other treatment.