

CHAPTER 1 INTRODUCTION

1.1 Background

Wounds are defined as the interruption of the continuity of body tissues by physical, mechanical, chemical, and thermal causes (Ariningrum, 2018). Wound healing is the process of restoring the normal structural function of the injured tissue. The body can overcome tissue damage itself but healing is relatively slow, which means that high nutrients are needed for the wound healing process (Sabale *et al.*, 2012).

Wounds can be classified based on the mechanism of injury such as incision wounds. An incision wound is one type of open wound or clean wound caused by a scalpel by minimizing skin damage (Mair *et al.*, 2013). After an injury occurs, the wound healing process will begin. Wound healing is divided into 3 phases, namely, inflammation, proliferation, and remodeling (Teoret, 2017).

Polymorphonuclear neutrophils (PMN) are part of the granulocyte white blood cell group that plays a role in inflammatory mechanisms. Polymorphonuclear neutrophil cells are the first cells to arrive at the site of inflammation that function as phagocytic cells because of their ability to efficiently destroy and engulf various pathogens (Jancinova *et al.*, 2011).

Fibroblasts are the dominant cells in the proliferative phase, fibroblasts play an important role in wound healing. Fibroblast cells proliferate, migrate to become more active in synthesizing matrix components and produce collagen which helps repair damaged tissue (Sumbayak, 2015).

According to Putri *et al* (2014), the most widely used antiseptic for surgery and wounds is Povidone-iodine. Although it is able to treat wounds, povidone-iodine has side effects that can irritate the skin and interfere with the wound healing process if its use is excessive (Dhivya *et al.*, 2015).

The World Health Organization (WHO) recommends the use of medicinal plants in the maintenance of public health, prevention and treatment of disease, medicinal plants in addition to relatively affordable prices for the community, easy to obtain and easy to use (WHO, 2013). One example of an effective medicinal plant is mung bean (*Vigna radiata* L.) which is believed to help cure various types of diseases. Mung bean, known as beans, has been consumed by many people as nutritious food and alternative medicine (Ganesan, 2018).

Mung bean seeds are known to contain flavonoid compounds that act as antioxidants, anti-inflammatory and anti-bacterial can accelerate the wound healing process (Moniharapon *et al.*, 2016). One way to facilitate the use of mung bean seeds is to make topical preparations in the form of ointment is a semi-solid preparation that was chosen as a dosage form because of its good stability, easy to use, able to maintain skin moisture, does not irritate the skin and has an attractive appearance (Ansel, 1989).

The effect of mung bean seed (*Vigna radiata* L.) extract ointment is thought to accelerate incisional wound healing on the total number of polymorphonuclear neutrophil cells (PMN) and fibroblast cells has not been scientifically revealed, so this study was conducted to determine the effect of giving mung bean (*Vigna radiata* L.) seed extract ointment on the total number of polymorphonuclear

neutrophil cells (PMN) and fibroblasts in incisional wound healing on albino rats (*Rattus norvegicus*).

1.2 Research Problem

Based on the background above, the formulation of my research problems is:

1. Can mung bean seed (*Vigna radiata* L.) extract ointment be able to decrease the total number of polymorphonuclear neutrophil cells (PMN) in incisional wound healing on albino rats (*Rattus norvegicus*) at day 14?
2. Can mung bean seed (*Vigna radiata* L.) extract ointment be able to decrease the total number of fibroblast cells in incisional wound healing on albino rats (*Rattus norvegicus*) at day 14?

1.3 Research Aim

1.3.1 General Purpose

The general purpose of this study was to determine the effect of mung bean seed (*Vigna radiata* L.) extract ointment in incisional wound healing on albino rats (*Rattus norvegicus*).

1.3.2 Special Purpose

The special purpose of this research are:

1. To determine the effect of mung bean seed (*Vigna radiata* L.) extract ointment to decrease the total number of polymorphonuclear cells (PMN) in incisional wound healing on albino rats (*Rattus norvegicus*).
2. To determine the effect of mung bean seed (*Vigna radiata* L.) extract ointment to decrease the total number of fibroblast cells in incisional wound healing on albino rats (*Rattus norvegicus*).

1.4 Research Benefits

1.4.1 Theoretical benefits

The theoretical benefit is to give information about the effect of mung bean (*Vigna radiata* L.) seed extract ointment in the incisional wound healing on albino rats (*Rattus norvegicus*) at day 14 and to provide data for further research purposes.

1.4.2 Practical benefits

The results of this study can be used as alternative medicine or other alternative therapies from natural ingredients that can help incisional wound healing.

1.5 Theoretical Base

When the skin is injured, the body automatically performs a wound healing process to repair the injured tissue. Wound healing generally consists of three processes that are interrelated, including: inflammation, proliferation, and maturation/remodelling (Gonzalez *et al.*, 2016).

The inflammatory phase is divided into two, namely the early inflammatory phase or hemostasis phase and the late inflammatory phase. The inflammatory phase lasts from the first day after wound formation until the fifth day. In the early phase of inflammation, hemostasis occurs. After a hemostatic response is achieved, polymorphonuclear neutrophil (PMN) cells get into the wound area. The role of polymorphonuclear neutrophil cells (PMN) is to remove dead tissue and bacteria by phagocytosis and prevent infection by pathogenic microbial agents. On the third day, the late inflammatory phase begins, and monocytes that differentiate into

macrophages get into the wound area. Macrophages have many roles in wound healing, which is to produce cytokines and growth factors that stimulate fibroblast proliferation, collagen production, and the formation of new blood vessels (angiogenesis) (Primadina *et al.*, 2019).

Fibroblasts are very important in the proliferative phase. Fibroblasts synthesize collagen as the main element for the formation of Extra Cellular Matrix (ECM) which is important in wound healing. Fibroblasts have several sequences in the proliferative phase, including epithelialization, fibroplasia, and wound contraction (Sumbayak, 2015).

The proliferative phase lasts from day 3 to 14. The purpose of this proliferative phase is to establish a balance between scar tissue formation and tissue regeneration (Primadina *et al.*, 2019). Epithelialization is the process of rebuilding the damaged skin layer. Keratinocyte cells that have migrated and differentiated into epithelial cells will migrate from the edge of the wound to the center of the wound and cover the wound surface. Fibroplasia is a process of fibroblast proliferation that results in the formation of scar tissue, the formation of scar tissue is due to the deposition of an irregular arrangement of collagen. In the wound contraction phase, the edges of the wound will be pulled towards the center of the wound caused by the activity of fibroblasts that differentiate into myofibroblasts thereby reducing the wound area. The force of wound contraction is caused by the actin coils in the myofibroblasts, and it is transmitted to the wound margins by cell-cell and cell-matrix bonds. Myofibroblasts are mesenchymal cells resulting from

degeneration of fibroblasts with functional and structural characteristics such as fibroblasts and smooth muscle cells (Sumbayak, 2015).

Ointments are semi-solid preparations intended for topical application to the skin. Ointment formulations require the presence of a base, the base itself is an inactive carrier of the topical preparation which can be liquid or solid which brings the active ingredient in contact with the skin. The ointment formulation uses a fatty ointment base in the form of a mixture of *adepts lanae* and *vaseline album* ingredients (Zulfa *et al*, 2015). The combination of the two bases can prolong the contact time of the drug on the skin thereby increasing the absorption of the drug and acting as a wound dressing (Andrie and Sihombing, 2018).

Based on the results of the study by Moniharapon *et al.*, (2016) showed that the ethanol extract of mung beans contains flavonoids and has anti-inflammatory, antioxidant and antibacterial activities. Tang *et al.*, (2019) explained that there is an interaction between inflammatory responses and oxidative stress. On one hand, inflammatory cells produce ROS which participates in oxidative stress; on the other hand, ROS can lead to an increased expression of inflammatory cytokines. Flavonoids can reduce the level of production of pro-inflammatory cytokines such as $\text{TNF-}\alpha$, IL-6, and IL-1 β which cause flavonoids to function as anti-inflammatory. Flavonoids can also give an antioxidant effect by inhibiting the excess production of ROS or directly scavenging ROS (Akhlaghi and Brandy, 2009). Flavonoids also function as antioxidants that can neutralize free radicals formed during the inflammatory phase (Evan *et al.*, 2006). Flavonoids can act as antibacterial by inactivating proteins on cell membranes so that bacterial growth

will be inhibited and prevent secondary infections (Larissa *et al.*, 2017). Flavonoid compounds in mung bean seeds have stronger antioxidant activity than their related plants, namely soybeans and the total flavonoid content of mung bean seeds is higher than soybean seeds (Fakhrudin *et al.*, 2020).

1.6 Hypothesis

Based on the research problem and the theoretical base, the hypothesis of this research is:

1. Mung bean seed extract ointment (*Vigna radiata* L.) can decrease the total number of polymorphonuclear neutrophil cells (PMN) in incisional wound healing on albino rats (*Rattus norvegicus*) at day 14.
2. Mung bean seed extract ointment (*Vigna radiata* L.) can decrease the total number of fibroblast cells in incisional wound healing on albino rats (*Rattus norvegicus*) at day 14.