Introduction

The skin is the largest organ of the body. It accounts for about 16 percent of a person's total body weight. It acts as primary immune defense against invasive pathogens and forms a protective barrier against chemical, mechanical, thermal and ultraviolet radiation damage. It also helps to regulate the body temperature. Impairment of this barrier, as in large mechanical traumas, burn wounds, and chronic wounds, leads to microbial infections and continuous inflammation, which in turn causes poor anatomical and functional outcome. So, the skin's ability to heal wounds quickly and effectively is essential for good health **(Morton and Phillips, 2016)**.

Wound healing is a complex and dynamic cascade that has attracted the attention of researchers for many years. It is a process essential for the restoration of the integrity and function of the damaged tissue as closely as possible to its normal state. Normal wound healing response begins the moment the tissue is injured. It includes several chemical mediators and growth factors and involves continuous cell-cell and cell-matrix interactions that allow the process to proceed in three overlapping phases, inflammation, proliferation and remodeling **(Rieger et al., 2014)**.

In developing countries, wounds constitute a major problem as the rate of severe complications is high and

financial resources are limited. This increases the popularity of natural therapy which represents the hope of finding a product with both higher efficacy and less cost for wound healing **(Gorski and Novella, 2014).**

Spirulina is a blue-green alga (cyanobacterium) that has been consumed as food since ancient times by the Mexicans and natives in the Lake Chad area. The alga is presently marketed as a food supplement (nutraceutical) due to its high contents of proteins, γ-linolenic acid, vitamins and minerals **(Wang et al., 2008)**.

Recent studies have demonstrated the antioxidant, antimutagenic, antiviral, anticancer, antiallergic, immune enhancing, hepato-protective, blood vessel relaxing and hypolipidemic effects of spirulina extracts **(Mani et al., 2008; Gershwin and Belay, 2008; Mala et al., 2009)**.

Its therapeutic implications in cases of diabetes, arthritis, anemia, cardiovascular diseases and cancer were reported **(Soheili and Khosravi-Darani, 2011; Hoseini et al., 2013**). Reviewing the literature, few studies were met with concerning the use of spirulina extract in skin wound healing **(Madhyastha et al., 2012; Gur et al., 2013).**

Aim of the Work

The present study was carried out to determine the effect of the spirulina extract on the healing process of skin wounds in adult male albino rats using light and scanning electron microscope.

Structure of the Skin

The human skin is composed of epidermis, an epithelial layer of ectodermal origin and dermis, a layer of mesodermal connective tissue. At the junction between the dermis and epidermis, there are dermal papillae which are projections that interdigitate with invaginating epidermal ridges. The hypodermis lies beneath the dermis. It is also called subcutaneous connective tissue, which stores adipose tissue and is recognized as the superficial fascia of gross anatomy **(Mescher, 2013)**.

Two major classes of skin are distinguished: thin skin, which covers the greater part of the body, and thick skin, which forms the surfaces of the palms of the hands, soles of the feet, and flexor surfaces of the digits **(Standring, 2008)**.

The epidermis of thin skin consists of a continuously self-renewing, four layered stratified squamous epithelium. The deepest layer is the stratum basale (germinativum layer), which contains the stem cells that proliferate to form all of the other cells of the epidermis. The cells of the stratum basale consist of cuboidal keratinocytes, melanocytes, and Merkel cells. Superficial to stratum basale is the stratum spinosum (prickle cell layer) where Langerhans cells are found along with many rows of spiny keratinocytes. The stratum granulosum (granular layer) lies just superficial to the stratum spinosum. It is

composed of one to three layers of flattened cells. These cells contain irregular and densely stained keratohyalin granules (Fig. I, **Standring, 2008**).

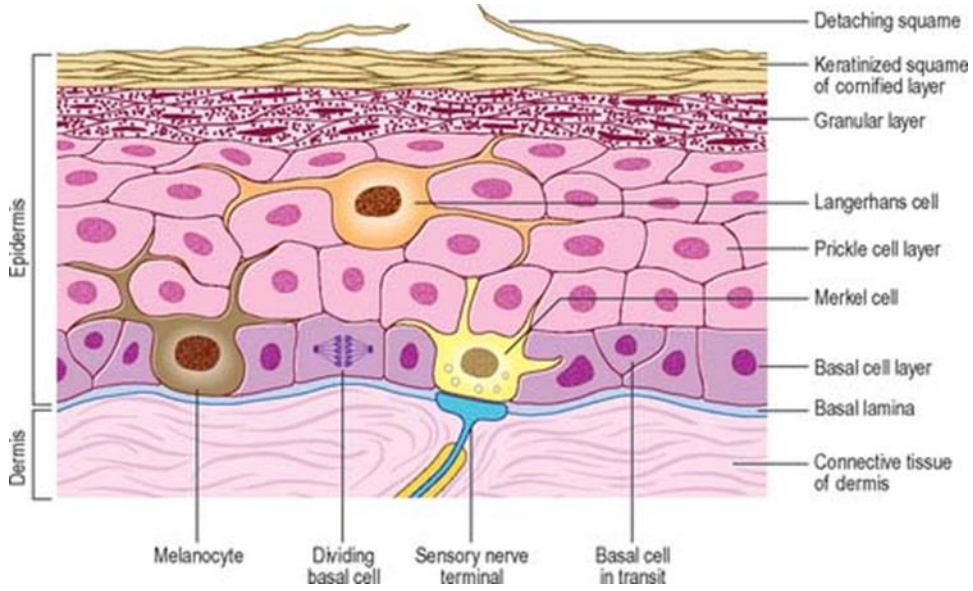

Fig. I: The main features of the epidermis, including different layers and cell types **(Standring, 2008)**.

The outermost layer of skin is the stratum corneum (cornified layer) which is formed of 15 to 20 layers of flattened, non-nucleated dead keratinocytes whose cytoplasm is filled with keratin. These cells are being constantly shed from the surface of the stratum corneum and being replaced by cells arriving from the deeper layers. In the thick skin of the feet and hands, there is a layer of skin superficial to the stratum granulosum known as the stratum lucidum which is made of several rows of clear, dead keratinocytes that protect the underlying layers **(Gartner and Hyatt, 2014)**.

The dermis is formed of two layers, the papillary layer and the reticular layer**.** The papillary layer is the superficial layer, formed of loose connective tissue containing collagen fibers type I, collagen fibers type III, elastic fibers, blood vessels and nerve endings. The reticular layer, lies deep to the papillary layer, is thicker and less cellular than the papillary layer (Fig. II, **Ross and Pawlina, 2016**). It is formed of thick, irregular bundles of type I collagen fibers and coarse elastic fibers. Thin skin contains hair follicles with their associated sebaceous glands and sweat glands. Each sebaceous gland opens into a hair follicle. Hair follicles, sebaceous glands and sweat glands extend through the whole dermis and may also be present in the hypodermis (**Ross and Pawlina, 2016)**.

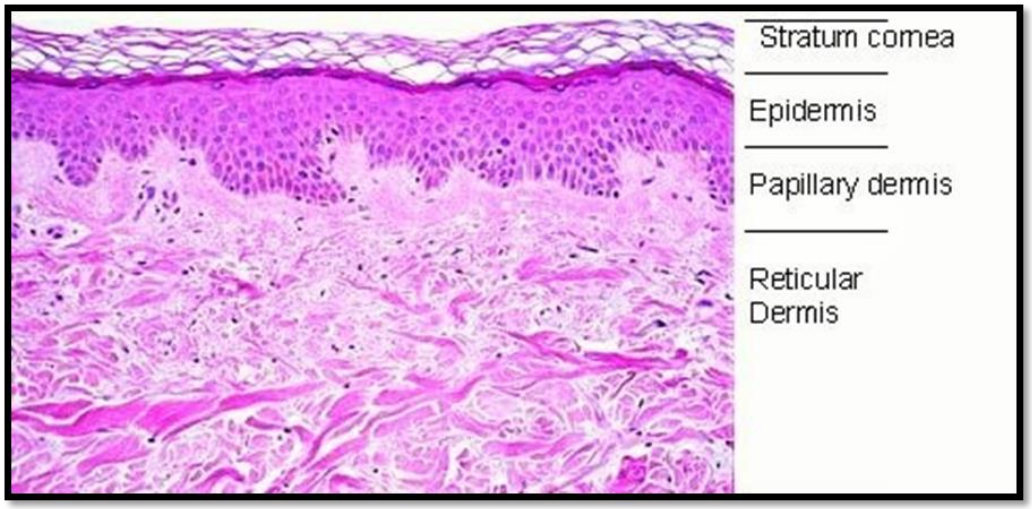

Fig. II: A photomicrograph of human skin showing the thin collagen fibers in the papillary dermis and dense collagen fibers in reticular dermis (**Ross and Pawlina, 2016)**.

The structure of the rat skin is similar to the human skin in many aspects. They share the presence of an epidermis, dermis and hair follicles. However, the skin of the rat deep to the dermis possess statrum adiposum, panniculus carnosus and startum fibrosum. The stratum adiposum represents the hypodermis in man while the stratum fibrosum represents the lower limit of the rat skin. Although, the human skin is adherent to the underlying structure the rat skin is loose. Also no sweat glands are observed in the rat skin **(Dorsett-Martin and Annette, 2008)**.

Skin Wounds

Any break in the continuity of the skin is called a wound. It is defined as disruption of normal anatomic structure and function **(Chard, 2008)**.

The nature of the wound and the manner in which it may heal are fundamentally linked to the mechanism of insult. Injuries caused by physical agents include: chemical, thermal, electrical injuries, mechanical traumas and injury caused by ionizing radiations **(Sharma et al., 2011).**

Kumar and Reddy (2014) described the mechanical injuries to take on a variety of forms, such as abrasions, contusions, lacerations, incisions, and excisional /puncture wounds.

Mechanisms of wound healing:

Wound healing is a complex and dynamic process involving soluble mediators, parenchymal cells, blood cells and extracellular matrix. Wound healing is classically divided into three phases: inflammation, proliferation, and remodeling. Considerable overlap exists between each phase, and a combination of cellular and biochemical events contributes to the wound healing process (Fig. III, **Park and Barbul, 2004**).

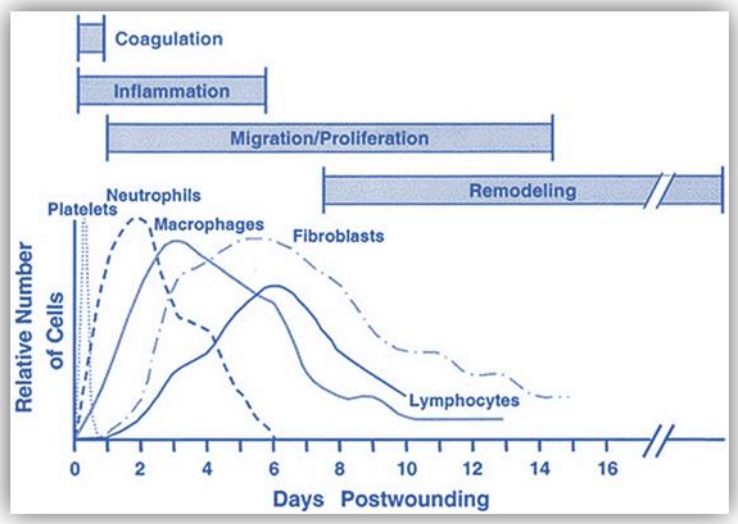

Fig. III: Timing of cellular involvement in different wound healing phases (**Park and Barbul, 2004**)**.**

Inflammatory phase (Day 0-5):

The normal healing response begins the moment the tissue is injured. As the blood components spill into the site of injury, the platelets come into contact with exposed collagen and other elements of the extracellular matrix. This contact triggers the platelets to release clotting factors as well as cytokines and essential growth factors such as platelet-derived growth factor (PDGF) and transforming growth factor beta (TGF-ß). So, finally a fibrin clot is formed **(Jacobson et al., 2008).**

Production of both kinins and prostaglandins leads to vasodilatation and increased small vessel permeability in the

region of the wound. Within 6 hours, circulating immune cells start to appear in the wound. The first inflammatory cells recruited are the neutrophils. They infiltrate massively the wound during the first 24 hours post-injury attracted by the numerous inflammatory cytokines produced by the activated platelets, endothelial cells, as well as by the degradation products from pathogens. Neutrophils enter apoptosis soon after infiltrating the wound and their numbers decrease rapidly after the third day **(Malech, 2007)**.

The release of cytokines during this apoptotic process is an important component in macrophage recruitment. Macrophages infiltrate the wound massively within 48-96 hours post-injury and reach a peak around the third day post-injury. They are involved in phagocytosis of neutrophils and in inflammatory cell recruitment (**Koh and DiPietro, 2011).**

Macrophages release a variety of cytokines and growth factors such as transforming growth factors, fibroblast growth factor (FGF) and PDGF, which induce fibroblast proliferation and extracellular matrix production. In this way, macrophages promote the transition to the proliferative phase of healing **(Brancato and Albina, 2011).**

Proliferative phase (Day 3-14):

The proliferative phase generally follows and overlaps with the inflammatory phase. The main focus of the healing

process in this phase lies in covering the wound surface (re-epithelialization), the formation of granulation tissue and restoring the vascular network (**Peterson, 2010)**.

The re-epithelialization process begins few hours after the wound formation. Keratinocytes from the wound edges migrate over the wound bed at the interface between the wound dermis and the fibrin clot. Re-epithelialization is complete in less than 48 hours in the case of approximated incised wounds, but may take longer time in the case of larger wounds where there is a significant tissue defect **(Santoro and Gaudino, 2005).**

Activated fibroblasts also migrate to the wound bed and form, with the macrophages, the granulation tissue. Fibroblasts first appear in significant numbers in the wound on the third day post-injury and achieve peak numbers around the seventh day post-injury. Fibroblasts produce large quantities of collagen which form the main constituent of the extracellular wound matrix **(Gurtner et al., 2008).**

Angiogenesis get activated by capillary sprouting. This occur on the second day post-wounding when endothelial cells from the side of the blood vessels adjacent to the wound begin to migrate in response to angiogenic stimuli including cytokines produced by platelets, macrophages and lymphocytes, low oxygen tension and lactic acid. Then, the newly formed capillary sprouts extend to the wound surface through which

blood begins to flow. This massive angiogenesis allows the supply of oxygen and nutrients necessary for the healing process **(Barrientos et al., 2008).**

Maturation and remodeling phase (Day 7 to 1 Year):

The last stage of the wound healing process includes a gradual involution of the granulation tissue and dermal regeneration. In this stage, the myofibroblasts which appear lately in the proliferative phase as a result of transformation of fibroblasts undergo apoptosis together with endothelial cells and macrophages. Granulation tissue is comprised of a large amount of collagen III, which is gradually replaced by collagen I that provides a higher degree of tensile strength (only 70 %). The high rate of collagen synthesis within the wound returns to normal tissue levels by 6-12 months, while active remodeling of the scar continues for up to 12 months after injury **(Toy, 2005).**

As remodeling progresses, there is a gradual reduction in the cellularity and vascularity of the reparative tissue which results in the formation of a relatively avascular and acellular collagen scar. In some cases shrinkage of the scar may give rise to an undesirable reduction in skin mobility resulting in contracture **(Oliveira et al., 2010).**

Wound closure:

Three different types of wound healing were described as primary, secondary and tertiary intentions.

Primary intention is the healing of a clean wound without tissue loss immediately following the injury and prior to the formation of granulation tissue. In this process, wound edges are brought together (re-approximated). Closure by primary intention will lead to faster healing and the best cosmetic result. It can only be implemented when the wound is precise and there is minimal disruption to the local tissue and the epithelial basement membrane as in surgical incisions **(Velnar et al., 2009).**

Secondary intention is implemented when primary intention is not possible. This is when the edges of the wound are far apart and cannot be brought together. This can be found in wounds of major trauma in which there has been a significant loss in tissue or tissue damage. The wound is allowed to granulate which results in taking longer time to heal and formation of broader scar **(Armitage and Lockwood, 2011)**.

Tertiary intention is also known as delayed primary closure. If the wound edges are not re-approximated immediately, delayed primary wound healing occur. This type of healing may be desired in the case of contaminated wounds. The wound is first cleaned and observed for a few days to ensure no infection is apparent, before it is surgically closed **(Velnar et al., 2009)**.

Factors affecting wound healing:

Multiple factors can lead to impaired wound healing. These factors can be categorized into local and systemic. Local factors (e.g. oxygenation, infection, venous insufficiency and foreign body) are those that directly influence the characteristics of the wound itself. Systemic factors (e.g. age, gender, stress, ischemia, diabetes or any immunocompromised conditions, alcoholism, smoking, medications and nutrition) are the overall health or disease state of the individual that affect the healing process **(Guo and DiPietro, 2010)**.

Oxygen level is a critical factor for nearly all wound healing processes. It prevents wound infection and stimulates angiogenesis. It increases keratinocyte differentiation, migration, and re-epithelialization. It also enhances fibroblast proliferation, collagen synthesis, and promotes wound contraction. In addition, the level of superoxide production (a key factor for oxidative killing pathogens) by polymorphnuclear leukocytes (PMNL) is critically dependent on oxygen level **(Bishop, 2008)**. In normally healing wounds, the temporary hypoxia after injury and the following hyperoxia increase the reactive oxygen species (ROS) which are thought to stimulate cell motility, cytokine action, and angiogenesis. Later increased levels of ROS cause additional tissue damage **(Rodriguez et al., 2008).**

Micro-organisms can impair wound healing by an increase in pathogenic effect due to production of toxins and destructive enzymes, release of free radicals, degradation of growth factors and secretion of immune-evasive factors. Infection also causes down-regulation of immune response, consumption of local oxygen, localized thrombosis and release of vasoconstricting metabolites **(Davis et al., 2008).**