



## Study the Effect of Hydrogen Peroxide and Alpha Lipoic Acid on Wound Healing in Rabbits

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### Abstract

*The aims of this study was to compare between the effect of hydrogen peroxide (oxidant) and lipoic acid (Anti-oxidant) on wound healing in rabbits. Fifteen adult healthy rabbits were used. It is divided in to three groups , control group, and hydrogen peroxide group(oxidant group) and hydrogen peroxide with lipoic acid group (Anti-oxidant group), also each group divide into three equal sub group for histopathological study at 1 week , 2 week and 3 week post operation. The wound was done in the femur (thigh) after general anesthesia by blade 3 cm. post-operation, in oxidant group, the animals given orally 50mg/bw of hydrogen peroxide daily for 7 days, 14 days and 21daysbut in antioxidant group, the animals were given orally 50mg/bw of hydrogen peroxide and 50mg/bw of Alpha Lipoic acid daily for 7days , 14days and 21days.*

*The histopathological examination appear in one week, both groups showed ulceration but in all groups showed.*

*In two week, control group and oxidant group showed of ulceration with dermal fibrosis. In two week, in anti oxidant group showed reduce the size ulcer with dermal fibrosis.*

*In three week, control group and oxidant group showed complete healing with moderate thickening of epidermis and hyperkeratosis with dermal fibrosis and reduce the size of ulcer. While in anti oxidant group showed complete healing with marked dermal fibrosis but presence of numerous of hair folliculate and absent of ulceration.*

*Conclusion the lipoic acid produced an increase the skin healing, which may be related to alteration of histopathological parameter and antioxidant activity.*

**Keywords:** Wound healing , oxidant , Anti-oxidant drugs and Alpha Lipoic acid.

### Introduction

A wound is a type of injury which happens relatively quickly in which skin is torn, cut, or punctured (an open wound), or where blunt force trauma causes a contusion (a closed wound). In pathology, it specifically refers to a sharp injury which damage the dermis of the skin<sup>(1)</sup>.

Wound healing is the process by which skin or other body tissue repair itself after trauma .in

undamaged skin, the epidermis (surface layer) and dermis (deeper layer) form a protective barrier against the external environment. when the barrier broken , an orchestrated cascade of biochemical events is set in to motion to repair the damage. This process is divided into predictable phases: blood clotting (hemostasis), inflammation, tissue growth (proliferation) and tissue remodeling<sup>(2)</sup>.

Lipoic acid (LA), also known as thioctic acid, is a compound present in small amounts in living organisms, which participates in several metabolic processes. LA has properties of metal chelating, reactive oxygen species detention, endogenous antioxidants regeneration and repair of systems<sup>(3)</sup>. Alpha lipoic acid, or thioctic acid, is a sulfur-containing fatty acid compound that acts as a co-enzyme and an antioxidant. It is called a "universal antioxidant" because of its potent ability to neutralize a wide range of free radicals<sup>(4)</sup>. Also LA has a wide range of benefits which includes the increase glutathione synthesis in animal tissues<sup>(5)</sup>. LA has been found to reduce the formation of glycosylated end products, or AGEs, AGEs are formed when protein reach with sugars and this process increase the risk of cardiovascular disease by oxidizing LDL cholesterol and making blood tough and inflexible<sup>(6)</sup>. Another benefit of LA is its ability to chelate, or bind with heavy metals. Both forms of alpha lipoic acid have been shown to form complexes with manganese, zinc, cadmium, lead, cobalt, nickel, and iron<sup>(7)</sup>. Alpha lipoic acid has a beneficial effect on cholesterol and lipid level, also ability to protect the liver from poisons and the ability to regenerate the liver if it has already been damaged. And it has been found to prevent calcium oxalate crystals or stones from forming and damaging the kidneys, also displays antihyperglycemic effects, systemic inflammation plays an important role in several diseases. Alpha lipoic acid has also been found to have significant benefits on inflammation due to its ability to down regulate pro-inflammatory cytokines. Also it used to improve mental function and might be a successful therapy for Alzheimers disease and other related dementias, alpha lipoic acid has used extensively in Germany for the treatment of diabetic neuropathy, a type of nerve damage that cause pain, loss of sensation and weakness<sup>(4)</sup>. It supplementation was able to accelerate wound repair in patients affected by chronic wounds. Alpha lipoic acid, in combination with hyperbaric oxygen therapy, down-regulate

inflammatory cytokines and growth factors, promoting the healing process<sup>(8)</sup>.

### Material and methods

In this study were used 15 from adult health rabbits were randomly selected, 5-month old, weighting 1.5-2 kg, were housed in clear wood cages and fed with vegetables and tap water under the same of laboratory environment. These animals divided into three groups: control group and oxidant group (hydrogen peroxide group) and antioxidant group (lipoic acid with hydrogen peroxide group). All the rabbits were anesthetized with intramuscular administration (I.M.) of 25mg/kg body weight xylazine hydrochloride and 15 mg/kg body weight ketamine hydrochloride (9). Make wound in the femur of the rabbit by scalpel after that give the rabbits systemic antibiotic (penicillin and streptomycin) for three days.

### Experimental Design

All the adult animals divided randomly in to three groups:

1-control group: contain 3 rabbits, and divided into three sub groups, post make the wound, and then divide to three period 7 days, 14 days, 21 days. At the end of all period, the specimens were taken from target tissue and fixed immediately in 10% formalin.

2-Oxidant group: contain 6 rabbits and divided in three subgroups:

The animals of this group were given orally 50mg/bw of hydrogen peroxide daily for 7 days, 14 days and 21 days., the specimens were taken from the target tissue include the skin, all the specimens of whole target were fixed immediately in 10% formalin for histological examination.

3-Antioxidant group: contain 6 rabbits and divided in to three subgroup; the animals of this group were given orally 50mg/bw of hydrogen peroxide and 50mg/bw of Alpha Lipoic acid daily for 7days, 14days and 21days and at the end of all period, the specimens were taken from target tissue and fixed immediately in 10% formalin.

### Microscopic examination

The histological sections were studied under the light microscope and the photographic pictures were taken for the sections of tissues by using microscope with camera (Leica DM 500 from Germany).

### Results

1-control group: in the one week , shows section of skin containing intense inflammatory infiltrate (INF), with desquamated tissue (DE) and hemorrhage (H) as in the image (1).

And in the two week period, complete repair with new epidermal lining in area of scar formation present generally prominent fibrosis in dermis. Area of scar there no hair follicles as in the image (2).

In the third week period scar formation and healed epidermis with scar formation in dermis above scab on the epidermis in the image (3B, 5A). Area of suppurative inflammation in the dermis and epidermis cover by scabas in image (4).

2-hydrogen peroxide group: in the one week period, there is moderate fibrosis in dermis , area erosion or loss of superficial epidermis in image (6A), but no evidence of infiltration of inflammatory cell. Another section in area of new growth of epidermal lining moderate fibrosis in the dermis but reduce or hardly any infiltration of inflammatory cells in image (5B). There is no ulcer.and in another section showed area ulceration with heavy infiltration of inflammatory cell extend to subcutaneous in image.

In the tow week period showed healed thicken epidermis, area of dermal scar, there no suppurative and there is no inflammation. The adjacent area with hair follicles and some of infiltration inflammatory cells in the dermis in image (7).

In the third week period , the epidermis cover the damage area dermal scar , in adjacent area there is scab formation and infiltration of inflammatory cells in the dermis in image (8).

3-lipoic acid and hydrogen peroxide group

In the first week period, there is evidence increase fibrosis associated with inflammatory cells in area of ulceration still present with heavy infiltration of mix of inflammatory cells mostly neutrophils and inflammatory process extend into the subcutaneous skeletal muscles. Another section increase dermal fibrosis in dermis with an area new formed epidermal lining indicated repair and no ulceration as in image(9).

In the tow week period showed small ulcer still present with scar formation in the dermis adjacent epidermis is thicken as in image (10).

In the third week period, complete recovery with numerous hair follicles in the dermis and area of thickened epidermis with heavy infiltration of inflammatory cells in the dermis also in adjacent in area of scar formation in the dermis as in image (11).

**Table (1)** scar formation.

groups\Times	Week 1	Week 2	Week 3
Control group	+	+	+
Oxidant group	+	+	+
Antioxidant group	+	+	+

**Table (2)** scab formation.

Group \ Times	Week 1	Week 2	Week 3
Control group	No present	No present	present
Oxidant group	No present	No present	present
Antioxidant group	No present	No present	No present

**Table (3)** Ulcer formation.

Groups \ Times	Week 1	Week 2	Week 3
Control group	Present	Present	Small ulcer
Oxidant group	Present	Present	Small ulcer
Antioxidant group	Present	Small ulcer	No present

**Table (4)** Hair follicles formation.

Groups \ Times	Week 1	Week 2	Week 3
Control group	=	=	=
Oxidant group	=	=	+
Antioxidant group	=	=	+

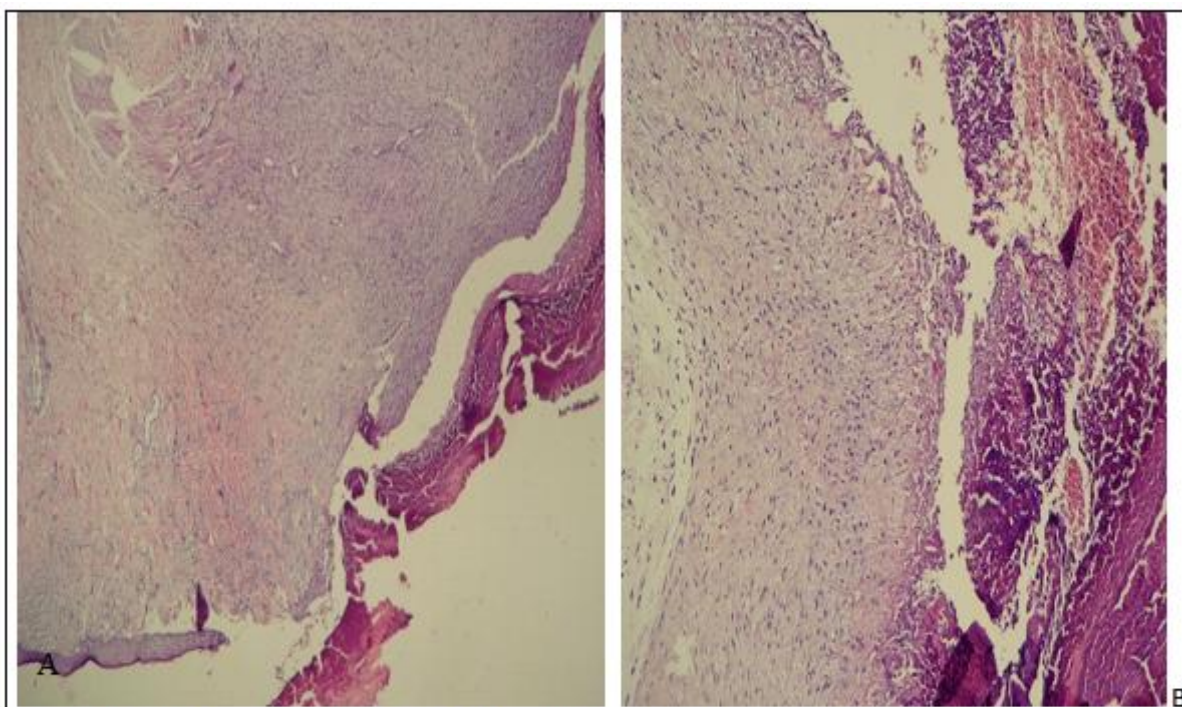
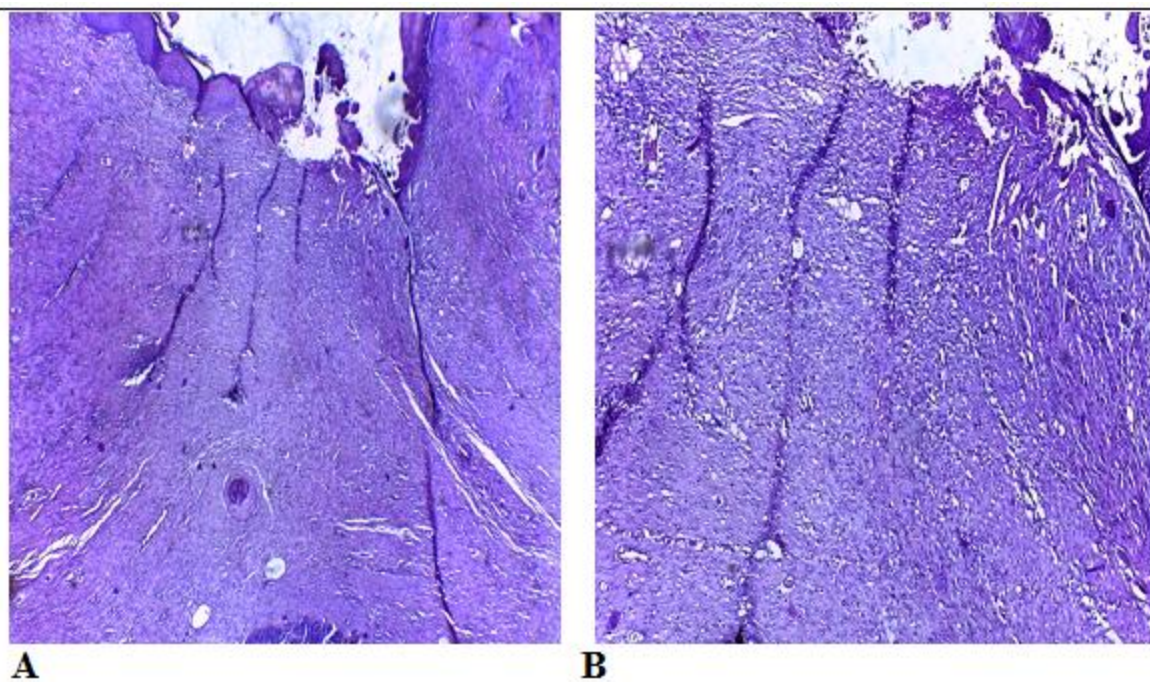
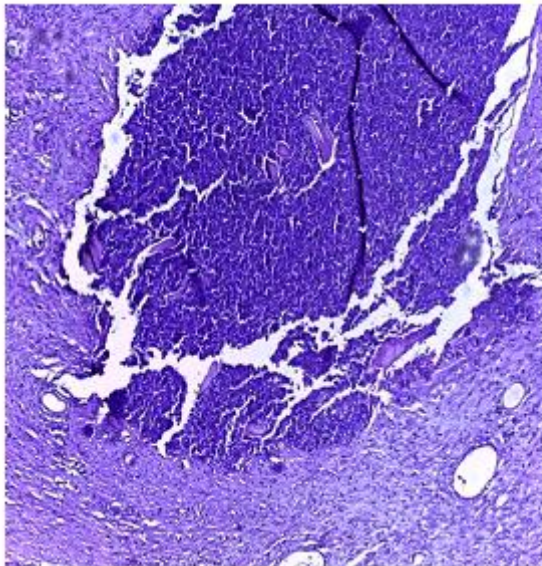


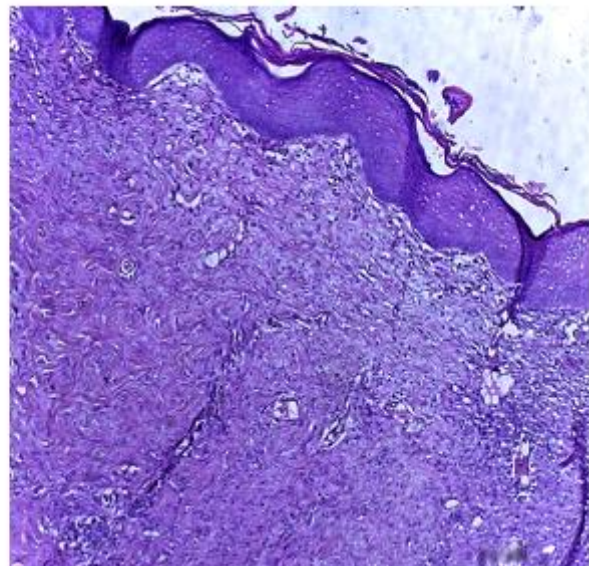
Image (1) shows section of skin of control positive animals reveals the site of surgical incision after one week containing intense inflammatory infiltrate (INF) , with desquamated tissue (DE) and hemorrhage (H) A) 50 X B) 125 X H&E



Image(2) shows section of skin positive animals reveals the site of surgical incision after two week containing scar formation and small ulcer . A)40X B)100X H&E

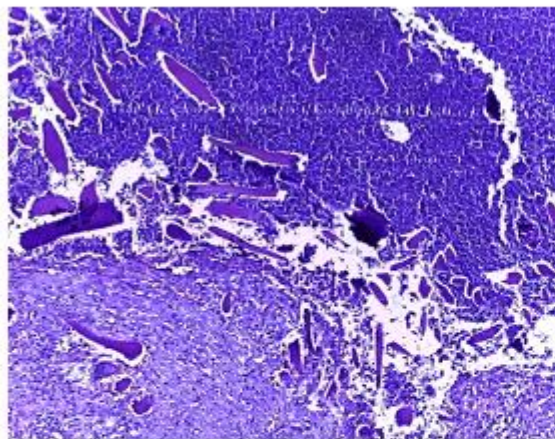


A

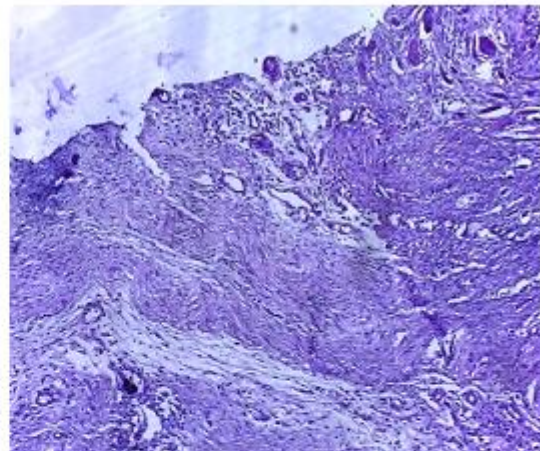


B

Image (3) shows section of skin positive animals reveals the site of surgical incision after two week containing formation abscessation in the dermis in figure(A) but in figure (B) shows section of skin animals in three week containing thickened epithelial lining . A)100X B)100X H&E



A



B

Image (4) shows section of skin positive animals reveals the site of surgical incision after three week containing formation abscessation in the dermis in figure(A) but in figure (B) shows section of skin animals in three week containing scab formation . A)40X B)100X H&E

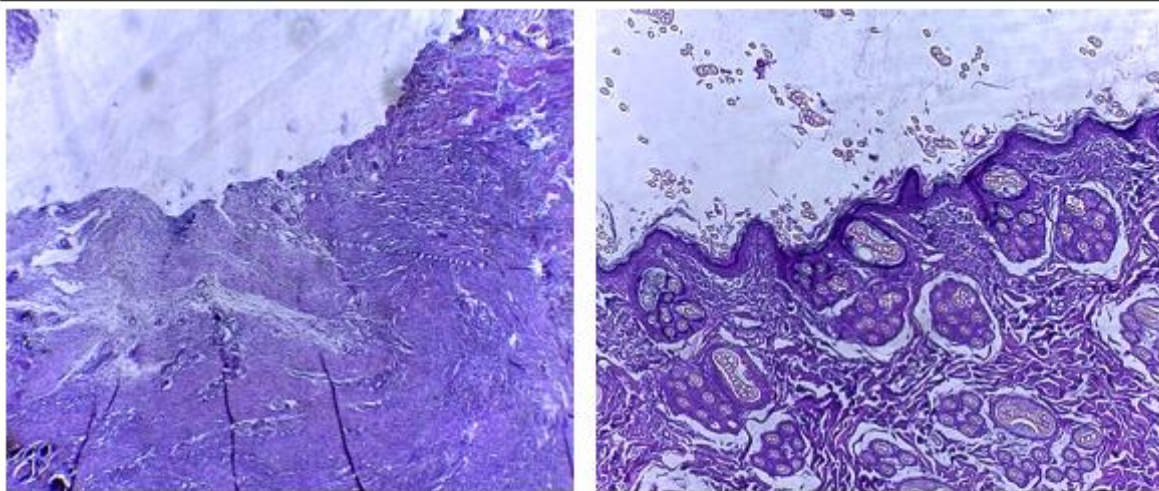
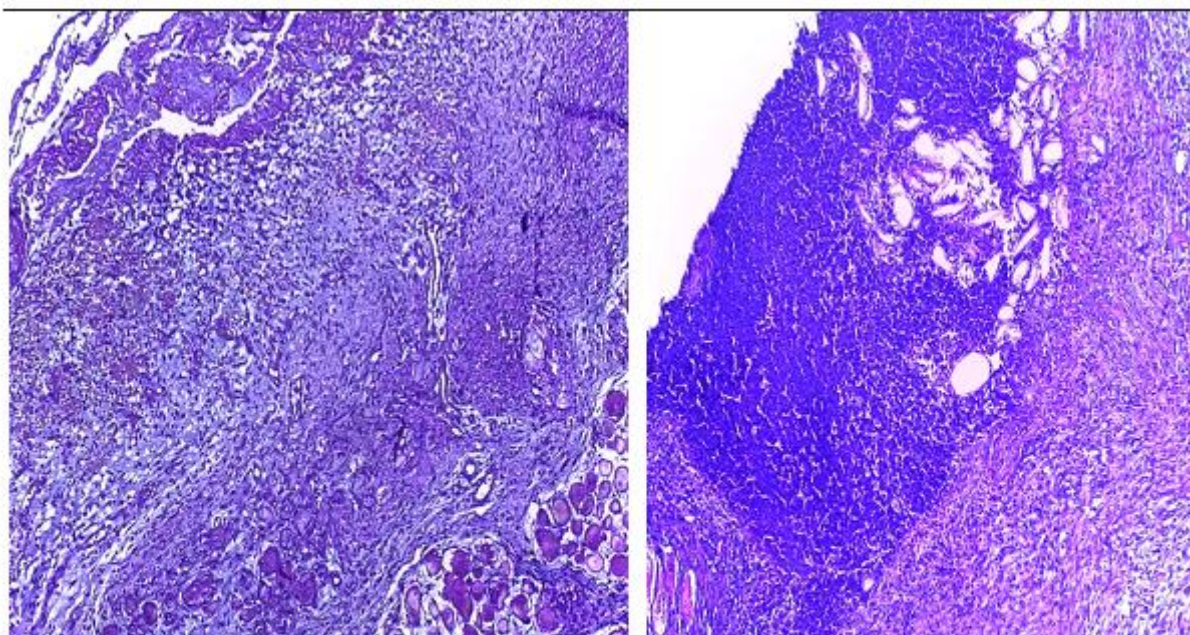
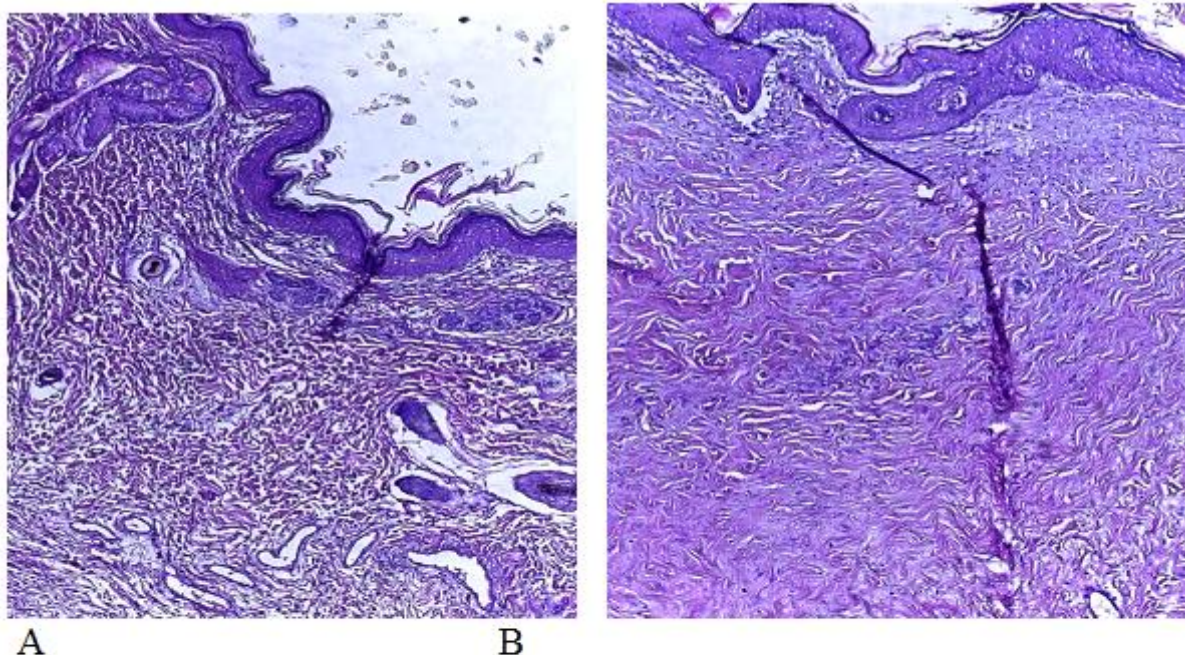
**A****B**

Image (5) shows section of skin positive control animals reveals the site of surgical incision after three week containing formation ulcer formation and scar in the epidermis and scab formation in figure(A) but in figure (B) shows section of skin (oxidant group) in one week containing number of hair follicles in dermis . A)40X B)100X H&E

**A****B**

Image(6)shows section of skin of (oxidant group) one week containing (A) ulcer and scar formation , (B) abscess formation with dermis. A)100 B)100 H&E.



Image(7) shows section of skin(oxidant group) two week containing thick epidermis lining and scar formation in figure A and B. A)100X B)100X H&E.

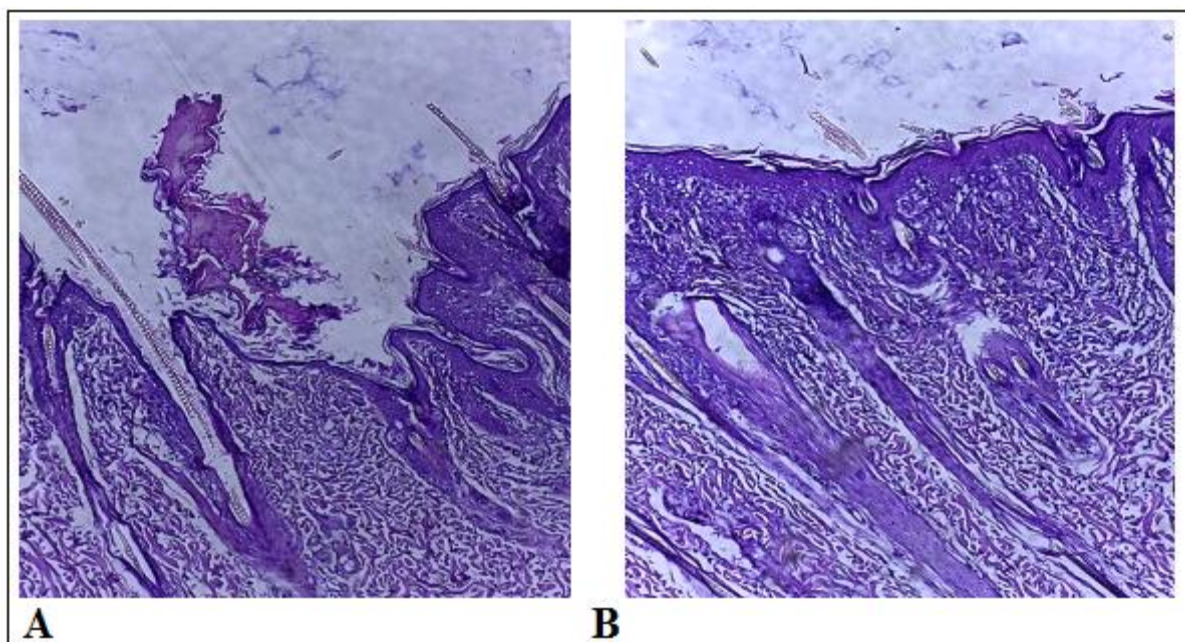


Image (8) shows section of skin (oxidant group) reveals the site of surgical incision after three week containing scab formation and dermal fibrosis with thinning epidermis in figure(A) but in figure (B) shows section of skin (oxidant group) in three week containing epidermis and dermal fibrosis . A)100X B)100X H&E

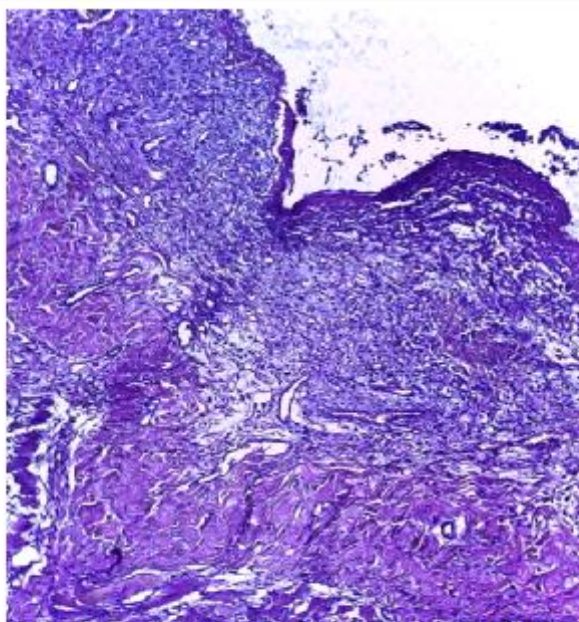
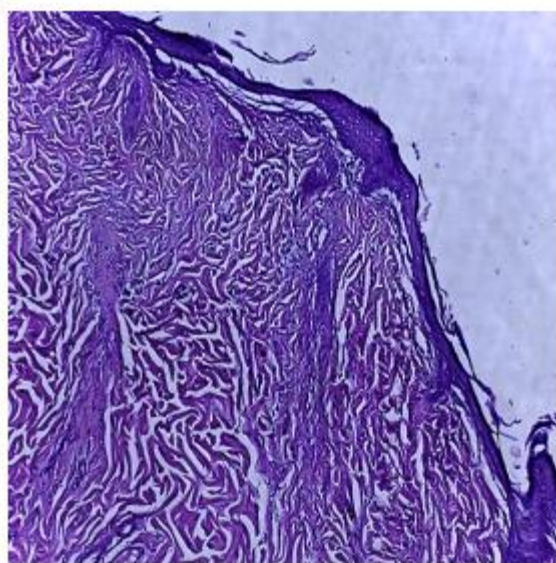
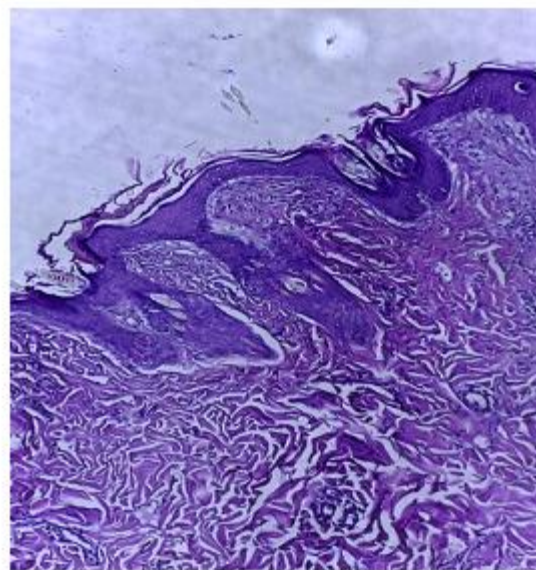


Image (9) shows section of skin(Anti-oxidant group) one week containing thin epidermal lining and fibrosis in the dermis. 100X H&E



**A**



**B**

Image (10) shows section of skin (Anti-oxidant group) reveals the site of surgical incision after two week containing epidermal lining with scar formation in figure(A) but in figure (B) shows section of skin (Anti-oxidant group) in two week containing thickening epidermis and dermal fibrosis . A)100X B)100X H&E



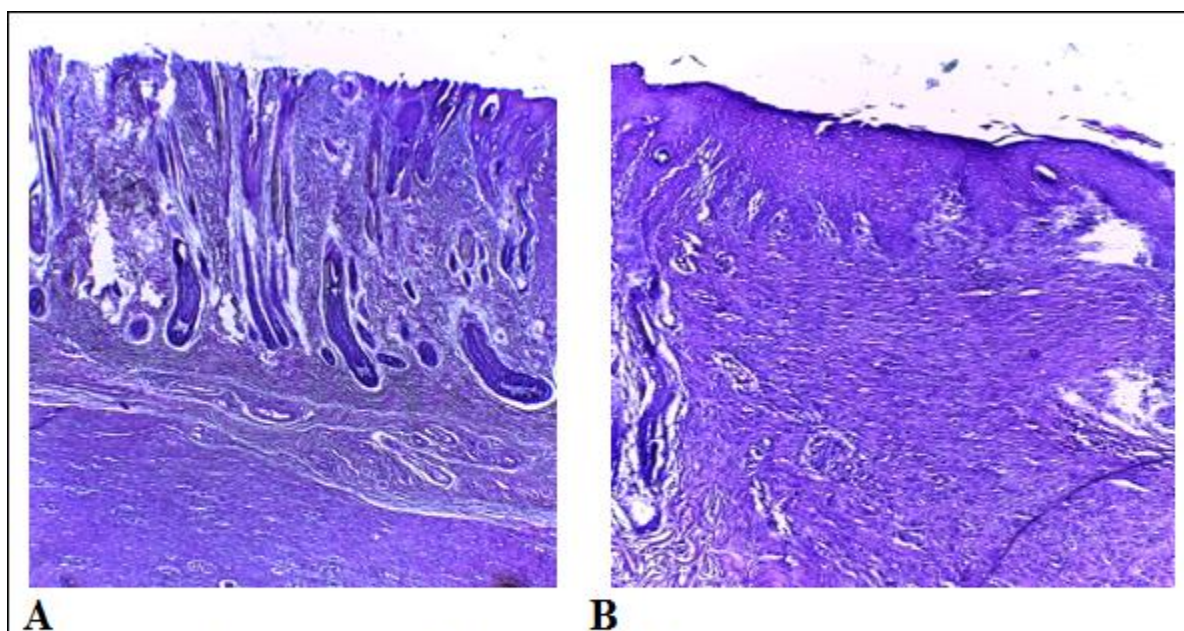


Image (11) shows section of skin (Anti-oxidant group) reveals the site of surgical incision after three week containing numerous of hair follicles in figure(A) but in figure (B) shows section of skin (Anti-oxidant group) in three week containing thickening epidermis and dermal fibrosis . A)100X B)40X H&E

### Discussion

In this paper we have proposed the use of oxidant and antioxidant as a useful, rapid and way to evaluate the effect of hydrogen peroxide and Alpha lipoic acid on wound healing based on the changes in the histopathological of the injured area.

Dermal fibrosis and epidermal thickening can result from increase in inflammatory cells infiltration or epidermal edema associated with enhanced blood flow.

In general, fibroblasts are known to be essential in the healing of tissue injuries including surgical wounds, the epithelialization and granulation tissue formation was created in the repair stage; fibroblasts begin to synthesize collagen and ground substances<sup>(10)</sup>.

In the initial stage of healing, hemostasis, increased vasodilatation in the surrounding tissue is necessary for healing to occur. However, simultaneous complications of vasodilatation have been found. In wounded tissue containing inducible nitric oxide synthase, vasodilatation could be over-stimulated which allow an excess concentration of neutrophils and cytokines to

develop<sup>(11)</sup>. These cells are capable of producing  $O_2^-$ , the superoxide radical, as defense mechanism against pathogen. The superoxide radical can be convert to hydrogen peroxide ( $H_2O_2$ ) which is capable of stimulates the production of other free radicals<sup>(12)</sup>. In addition nitric oxide synthase stimulates the production of nitric oxide. The substance is capable of producing oxynitrite, a harmful oxygen free radical that increase the oxidative stress and escalates damage to the tissue<sup>(11)</sup>. All of these factors contribute to the wound site becoming dense with free radicals and the cascade effect of tissue damage<sup>(12)</sup>.

The healing of skin wound is a complex and dynamic phenomenon of cellular structure restoration in injured tissues, in which multiple mechanisms and mediators are involved. the role of substances with antioxidant properties in this scenario appears to be still controversial, some authors suggest that the excess of reactive oxygen species may slow the healing process. Probably by causing cell membrane lysis. moreover, as discussed by other authors, a microenvironment at the lesion site constituted by factors such as reactive oxygen species during the early stages of

healing. besides acting as antibacterial agents , could contribute to healing, by influencing different phenomena such as hemostasis, inflammation, vascularization, and reepithelialization. according to the latter idea , the pro-oxidative activity of lipoic acid observed in vitro assay in this study could be correlated to its ability to produced local cellular stimuli that would result in greater tissue re- epithelialization in earlier phases, possibly resulting in higher healing rate abserved on the day 7 after surgery on the in vivo assay<sup>(3)</sup>.

The antioxidant eliminates peroxy radicals and protects the cell membrane from oxidation .this antioxidant can also protect collagen and glycosaminoglycans from oxidation, which may speed the rate of wound closure. ALA is antioxidant that function to protect the cell from lipid peroxidation by eliminating reactive oxidant species (ROS), Alpha lipoic acid effect direct on free radicals, and also acts to regenerate vitamin E, vitamin C, glutathione, co enzyme Q10. Antioxidant consumed upon the infiltration of free radicals in order protects unsaturated fatty acids in the cell membranes from oxidation<sup>(13)</sup>.

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