COMPARISON BETWEEN EGGSHELL MEMBRANES AND BURN CARE SHEETS FOR TREATMENT OF SECOND DEGREE BURN IN WISTER RATS MODEL

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ABSTRACT

Burn is one of the main reasons of death and healing, burn is a quite competition in modern medicine. In the present study, the healing effects of eggshell membranes compared with burn care sheets were investigated on second degree burn in rats. Thirty Wister rats were used in this study. The rats were randomly divided into three groups. After making of standard second degree burn injury on the back of the animals, two treatments were investigated in two groups. Group one implanted with eggshell membranes which covered with bandages while the second group dressed with Fusidic acid, and group three received no treatment. Curative was evaluated by the rate of wound healing. Histopathological assessment of injury samples was also achieved. The use of egg shell membrane improved the rate of wound contraction when compared with fusidic acid application and control group. Additionally, histopathological evaluation established these results. The results of this study indicated that the use of egg shell membrane in the healing of wound in second degree burned rats has the potential and it is better than fusidic acid group and control one with better healing cascades, The procedure of epithelialization was enhanced in the patient skin cured with eggshell membranes due to its efficacy in antimicrobial and cell proliferating effects.

Key words: Eggshell membrane, burncare sheet, burn, Wister rat

Introduction

In fact, the burning of tissues is a complicated process, involving a dynamic set of events including clotting, bleeding, inflammation, epithelialization and tissue remodeling, which are caused by serious injury and is related to infection, damage, discomfort and even death (Garcia *et al*., 2010).

Topical cure of burn is very imperative due to the burn reflects as the most important life threatening issues and different agents have been suitable for this purpose, some of them have been used for a long period, the ideal agent, which applied topically, may cure the burn in a short time with best squeal, diminution the mortality and morbidity by prevention of sepsis and bacterial contamination and can be retrieved easily and cheap (Bayat *et al*., 2006).

The broad antimicrobial activity of the cream is silver sulfadiazine 1% per cent; bactericidal for a many gram negative and gram-positive bacteria and effective against yeast. Some studies have shown that this cream should not be used for a longer period of time for extensive injury due to retarded wound healing and negative reactions and side effects such as resistance and leucopenia (Hussein *et al*., 2017).

The avian egg shell is a shell and membrane structure that constitutes about 10% of the egg weight. The coating consists primarily of calcium carbonate ($CaCO_3$) (95%) and an organic matrix of proteins, glycoproteins and proteoglycans (90% and 3.5 per cent) (Jalil *et al*., 2020; Mann *et al*., 2006).

The shell is composed of calcitic columns of two parts: a major part with bumpily parallel sides (the palisade layer) and a slight part making the rounded inner ends (mammillary cone layer) that are close to the fibers of the shell membranes. The outermost sheet of the chicken eggshell is the cuticle, made by vesicles that are >85% proteinaceous (Burley and Vadehra, 1989). The eggshell sheaths are found between the egg white and the calcified shell, and are thought to play an significant role in defining the origin and ultrastructure of the calcified shell (Nys *et al*., 2004; Arias *et al*., 1991).

Collagen fibres of the eggshell sheath are structured into two specific membranes: a thin inner one situated on the egg white (albumen), and a thick outer membrane attached to the shell. Either eggshell membrane contains cross-linked collagens (I, V and X), glycosaminoglycans (GAGs), egg white proteins (*i.e*. Ovotransferrin, Lysozyme) and eggshell matrix proteins (*i.e*. Ovocalyxin-36). Hydroxyproline, hydroxylysine and desmosine are detected in its acid hydrolysates (Hincke *et al.,* 2010).

Eggshell and eggshell membrane are a major by-product of the industry of egg production (Ahmed *et al.,* 2019). Many studies have been accepted to apply eggshell waste for potential value added applications (Cordeiro and Hincke, 2011). Furthermore, eggshell membrane is an organic material that has been revealed to raise cellular activity and collagen production, also it has been recommended as skin moisturizer, wound recovery, skin growth (Park *et al*., 2012). Either they found calcium carbonate nanoparticles (CCNPs) synthesized from egg shell, safe with height dose on the animal brain (Ghaji *et al*., 2020).

In this study, we investigated the anti-inflammatory, antimicrobial activities of eggshell membrane when compared with burn care sheets for treatment rats burn.

Materials and Methods

Ten hen´s eggs were brought from local market, washed thoroughly with distilled water twice. The air chamber was opened and the contents were discarded, acetic acid solution 10% was added in the empty eggs and left for 6 hours. The eggshell membranes were manually isolated from the eggshell, washed with distal water and kept in containers filled with deionized water which preserved at 4ºC until use

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(Guarderas *et al*., 2016).

Thirty Wister rats, their weights ranged 210-230 g of both sexes were used in the study under standard environment. These rats divided randomly into three groups. 2x2 cm^2 area in the back regions were clipped, shaved and prepared for surgery. Each animal anesthetized with combination of xylazine 21 mg/kg and ketamine 45 mg/kg i.m. Hot plate 2x2 cm^2 at 100ºC for 30 seconds was used to induce second degree burns (Fig.1,2).

The first group implanted with eggshell membranes which covered with bandages while the second group dressed with Fucin (Alexandria Co. for Pharmaceuticals and Chemical Industries, Alexandria- Egypt) which contained gauze dressing impregnated with sodium fusidate (sodium salt of fusidic acid) in white soft paraffin and covered with bandages and the third group had no any treatment for their burns and regarded as control group. The burns were studied macroscopically with camera photos (Sony Digital Camera) and biopsies were taken after 7, 14, 21 days for histo-pathological examinations.

Fig.1: Burning of skin with hot plate

Fig.2: The burnt site

Fig.3: The burn site of group 1 after 21 days

Fig.4: The burn site of group 2 after 21 days

Fig. 5: The burn site of group 3 after 21 days

Results and Discussion

No mortality was noticed in the rats during whole period of the study.

Macroscopic evaluation

The results of the study in the five days had changes for all groups of study. The burn site of G1 (egg shell) after 21 days showed complete healing with few scar tissue as in Fig. 3, while the burn site of G2 (burn sheet) after 21 days showed profuse scar tissue as in Fig. 4 and the burn site of G3 (control) after 21 days showed weak healing with very profuse scar tissue as in Fig. 5.

Microscopic evaluation

The results in first group that covered by egg shell after the one week show partial destruction of superficial skin layers dermal layers as well as, mild inflammatory change like slight oedema and engorgement of blood vessels (Fig. 6), at same period on burn sheet groups, show inflammatory changes with sign of coagulation and infiltration of inflammatory cells (Fig. 7). While in control group it showed wide destruction of superficial skin layers, either show inflammatory changes with sign of coagulation and infiltration of inflammatory cells in dermal layers (Fig. 8).

After fourteen days of exposure all animals to the diverse treatments, the results in first groups shows regeneration of epidermis with mild inflammation (Fig. 9, 10). The second group, which treated with burn sheet of Fucidic acid as clarified a partial destruction of superficial skin layers, inflammatory

changes with sign of coagulation in dermal layers as well as, there are slight oedema and which depicts mild inflammation (Fig.11,12).On the other hand the third group showed partial regeneration of epidermis, but the dermis layer still affected and suffering from oedema in a burn skin tissue (Fig.13).

In third week of treating first group of egg shell seemingly recorded an improved healing process when it denoted in (Fig.14), depicts complete regeneration, normal skin layers by formation of new blood vessels and profuse fibrosis and hyperplasia of epidermal layers with a new formation of hair follicles, profuse collagen network and mild infiltration of inflammatory cells (Fig.15) at the same time in groups of burn sheet, skin of rat shows almost complete regeneration of skin layers, newly formed blood vessels and profuse of irregular collagen network fiber (Fig.16, 17). In third group, show complete regeneration of epidermis, but the dermis layer still affected and suffering from oedema and infiltration of inflammatory cells and scanty collagen fiber with irregularity (Fig.18).

Burn restoration is a communicating process, depend on the organization between extracellular matrix components, different cell types and their soluble mediators. Stimulators of

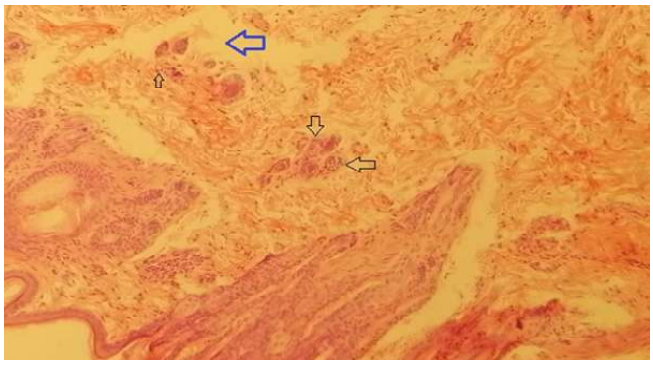

Fig. 6: Egg shell-groups one week skin of rat shows histology of burned skin show partial damage of superficial skin layers, inflammatory changes with sign of coagulation in dermal layers as well as, there are slight oedema (blue arrow) and engorgement of blood vessels (black arrows). H&E, X100.

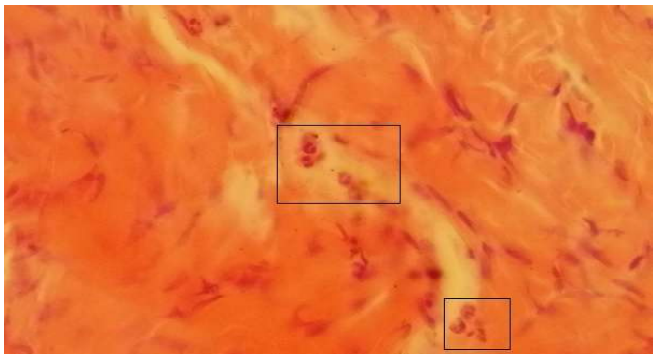

Fig. 7: Burn sheet-groups one week. Deep second- degree burn on the rat infiltration of inflammatory cells (black boxes) in the dermis. H&E, X400.

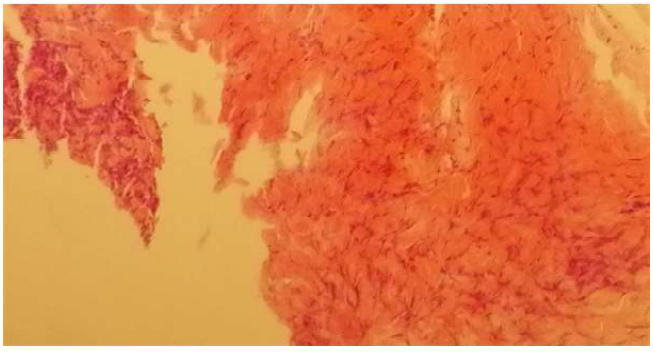

Fig. 8: Control groups one week. Deep second- degree burn on the rat skin shows widespread destruction of superficial skin layers, inflammatory changes with sign of coagulation in dermal layers with partially survived skin appendages, epidermis exfoliated. H&E, X100.

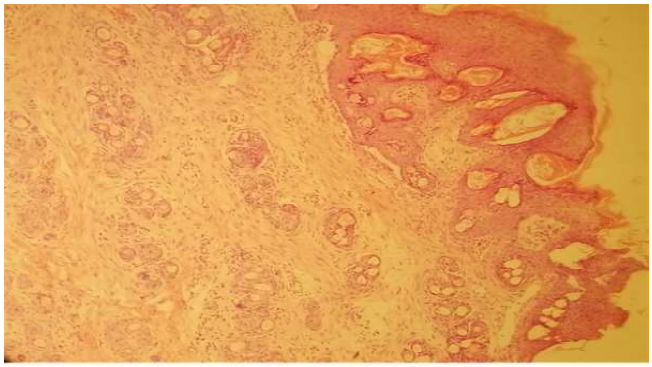

Fig. 9: Egg shell group's two weeks. Skin of rat shows burn skin tissue treated with egg shell membrane which represents regeneration of epidermis on day 14. H&E, X100.

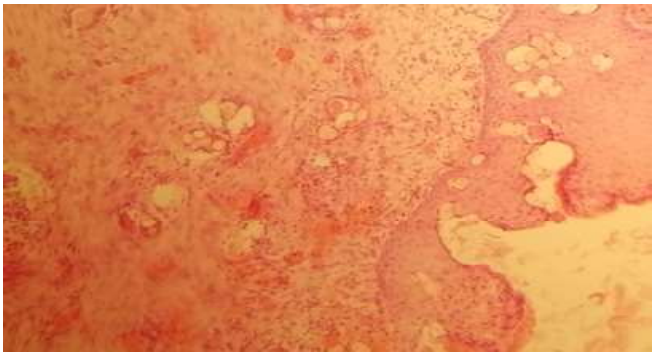

Fig. 10: Egg shell groups two weeks. Skin of rat shows burn skin tissue treated with egg shell membrane which depicts mild inflammation and regeneration of epidermis on day 14. H&E, X100.

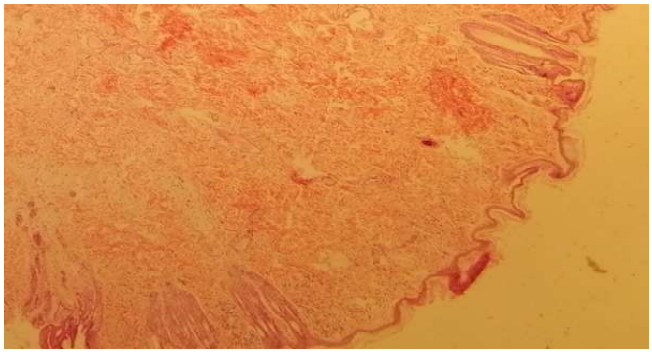

Fig. 11: Burn sheet groups two week. skin of rat shows histology of burned skin illustrate partial destruction of superficial skin layers, inflammatory changes with sign of coagulation in dermal layers as well as, there are slight oedema. H&E, X10.

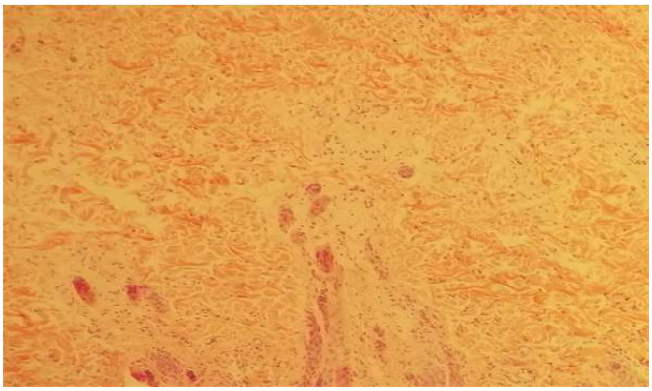

Fig. 12: Burn sheet groups two weeks. Skin of rat shows burn skin tissue treated with burn sheetwhich depicts mild inflammation and regeneration of epidermis on day 14. H&E, X100.

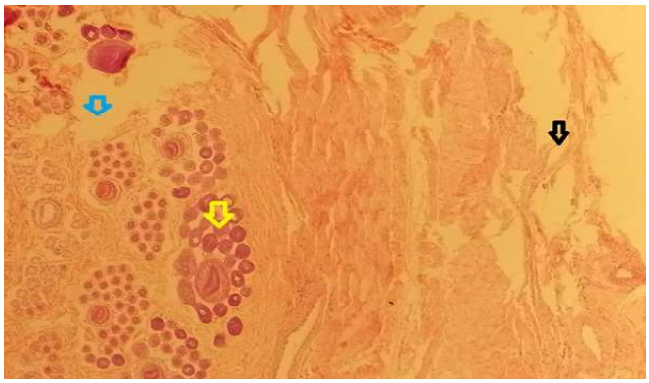

Fig. 13: Control groups two weeks. Skin of rat shows burn skin tissue partial regeneration of epidermis (yellow arrow), but the dermis layer still affected and suffering from oedema on day 14. H&E, X100.

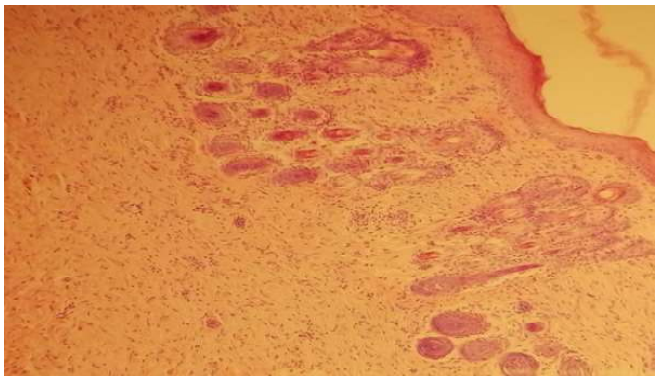

Fig. 14: Egg shell groups three week. Skin of rat shows completely normal skin layersby formation of new blood vessels and profuse fibrosis and hyperplasia of epidermal layers with a new formation of hair follicles. H&E, X10

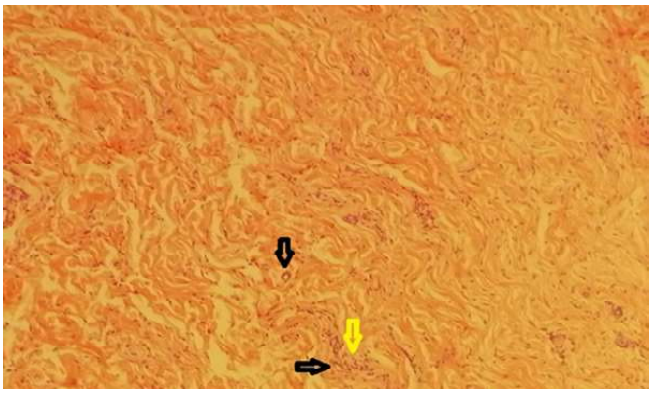

Fig. 15: Egg shell groups 3 week: Skin of rat shows dermis tissue treated with egg shell membrane which depicts complete regeneration, with newly formed blood vessels (black arrows) with profuse collagen network and mild infiltration of inflammatory cells (yellow arrow). H&E, X40.

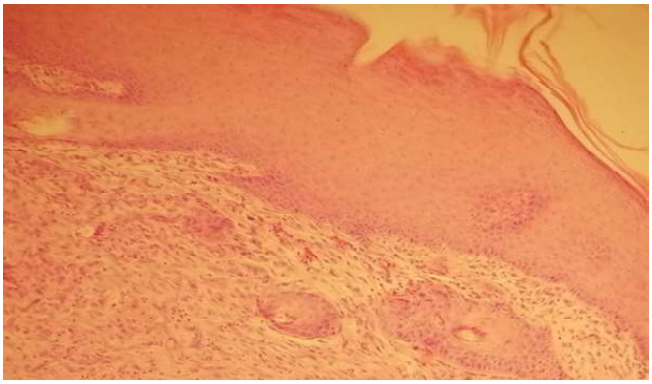

Fig. 16: Burn sheet groups three week. Skin of rat shows almost complete regeneration skin layers. H&E, X10.

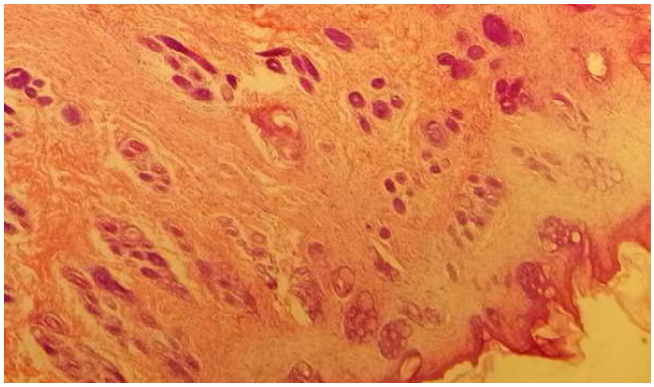

Fig. 17: Burn sheet groups three week: Skin of rat shows burn skin tissue treated with burn sheet membrane which depicts almost complete regeneration with newly formed blood vessels (black arrows). H&E, X100.

this process are highly desirable as requirements in burn healing products. Eggshell membrane is a biomaterial which is intended for therapeutic use for this purpose (Vuong *et al*., 2017).

Emergence of fucidic acid resistance led to restrict use of this drug as monotherapy for longer duration to treat chronic skin disorder as well as possible adverse drug reactions and increase in the cost of therapy (Musmade *et al*., 2013) and this accord with this study which showed a professed scar formation in the group 2 which treated with fucidic acid.

It was perceived that chondroitin sulfate, glucosamine, collagen and hyaluronic acid isolated from eggshell membrane showed anti-inflammatory properties based on decrease of pro-inflammatory plasma cytokine levels, rise cellular activity and collagen production (Park *et al*., 2012; Long *et al*., 2008a) and these findings are agreed with the present study which revealed that there is a complete regeneration of burned skin with mild infiltration of inflammatory cells when

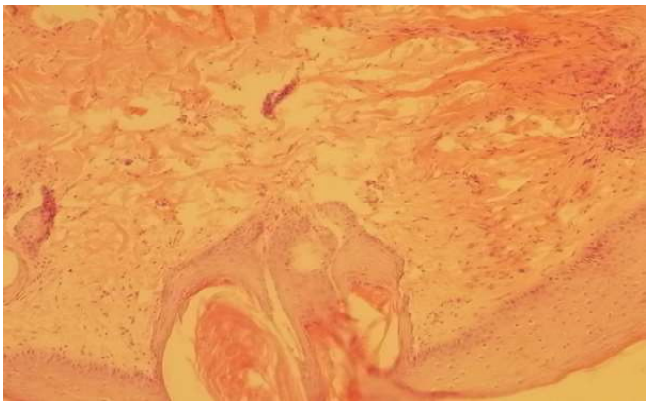

Fig. 18: Control groups three weeks. Skin of rat shows burn skin tissue complete regeneration of epidermis, but the dermis layer still affected and suffering from edema and infiltration of inflammatory cells and scanty collagen fiber with irregularity on day 21(black arrows). H&E, X10.

treated with egg shell membrane.

The mild inflammation, regeneration of epidermis and slight edema of first group are compatible with (Arias *et al*., 2007; Zadik, 2007) they are described that fresh eggshell membrane can be used as an anti-microbial tissue adhesive to reliefcuring of certain skin lacerations.

The complete healing of burned skin which treated by egg shell membrane when compared with the fucin group or control one due to the eggshell sheaths are a prospective source of proteoglycans, glycosaminoglycan's and bio-active proteins which are related with the fibers collected of collagen types I, V and X. These combinations are present in eggshell membrane in significant amounts compared to other known sources (Long *et al*., 2008b).

The usage of an egg membrane seems to be safe formula of burn treatment and as a possible application for this therapy, the egg membranes could be incorporated into dressing and used with skin grafts. Or may use as powder considered as a respectable biocompatible graft material to healing a critical bone gap moreover, this treatment might be recommended for emergency field medicine (Atiyah *et al*., 2020). In conclusion, the using of egg shell membrane in the healing of wound in second degree burned rats is better than fucin group and control one with better healing cascades. The method of epithelialization was enhanced in the patient skin treated with eggshell membranes due to its efficiency in antimicrobial and cell proliferation effects.

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