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Evaluation of Topical Application of Propolis, Black Seeds and Honey on Oral Mucosal Healing in Rabbits (Histological and Immunohistochemical Study on TGF-β3)

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Abstract: ***Background**: Wound healing is classically characterized by the transient development of granulation tissue that supports rapid proliferation, migration, and differentiation of the adjacent epithelium. The transient reactive stroma includes vascularization of the wound, infiltration of inflammatory cells, and differentiation of the dermal fibroblasts. Growth factors released in the traumatized area stimulating the growth of epithelial cells and fibroblasts, initiate the formulation of new blood vessels, and Improved glucose level. The aim of the present study was to evaluate effect of mixture of propolis, black seed and honey on oral wound healing. **Materials and methods**: Twelve New Zealand male rabbits were used in this study, they were divided into three groups according to 3, 7, and10 days healing intervals (4 animals for each group).All animals were subjected to alloxan injection to induced diabetes which was controlled by insulin. Application of a mixture of propolis, black seed and honey was done at wound of right side of check mucosa (Experimental), whereas left wound site (Control) was left to heal spontaneously. Histological and immunohistochemical study on TGF-β3, assessment was performed for all groups. **Results**: Histological and immunohisto chemical findings of this study showed that re-epithelialization,and remodeling of dermal fibrous connective tissue were accelerated after topical application of a mixture of propolis, black seed, and honey at wound site supported by the positive expression of TGF-β3 by the cells at wound site. **Conclusion**: Topical application of a mixture of propolis, black seed and honey was effective in wound healing of controlled diabetics.*

Keywords: oral mucosa, propolis, black seed, honey. alloxan –induced diabetes TGF-β3

1. Introduction

The reconstruction of the damaged tissue requires the coordinated action of a large number of biochemical systems, the nature of which depends on the presence or absence of contaminating toxins in the wound (1).

Diabetes mellitus is a common and serious metabolic disorder associated with many functional and structural complications. It is one of the most frequently diagnosed endocrinopathies on humans. Improved glucose level control with insulin injections and oral medications have allowed for the diabetic population to live longer and healthier lives (2).

The word propolis is derived from the Greek, pro-, for/or in defense & polis-, the city, that is: defense of the city (or the hive) (3).It is a sticky, resinous substance collected by honey bees from the sap, leaves, and buds of plants, and then mixed with secreted beeswax (4). It has been characterized variously as an anti-bacterial, anti-viral, anti-inflammatory, anti-oxidant, and anti-carcinogenesis agent(5), reported the propolis is capable of stimulating the production of (TGF-β3) (6).

Nigella Sativa (black seed) is an annual flowering plant, native to southwest Asia. The seeds of Nigella sativa, commonly known as black seed or black cumin, are used in herbal medicine all over the world for the treatment and prevention of a number of diseases and conditions. The seeds/oil has anti-inflammatory, analgesic, antipyretic, antimicrobial and antineoplastic activity. The seeds are characterized by a very low degree of toxicity. It would appear that the beneficial effects of the use of the seeds might be related to their cytoprotective and antioxidant actions, and to their effect on some mediators of inflammation **(7).**

Topical application of honey and black seed to wounds has been found to enhance wound healing **(8).**

Growth factors are biologically active mediators that bind to specific receptors on target cells and regulate genes involved in cell growth, wound healing and regeneration. The expression of these receptors is thus fundamental importance for the response of the cells to the factors **(9).** Pleiotropic and redundant functions of the TGF-β3 family concern control of numerous aspects and effects of cell functions, including proliferation, differentiation, and migration in all tissues of the human body **(10).**

2. Aim of the Study

Study the effect of topical application of mixture of propolis, black seed and honey on healing of oral mucosa of controlled alloxan-induced diabetes rabbits by means of histological and immunohistochemical analysis on TGF- β3.

Materials and methods

- Alloxan(100 mg, England)
- Insulin (0.1mg/kg B.W).
- Propolis(10 gm),Black seed (10 gm), Honey(20 ml),
- Ketamine hydrochloride 50mg and Xylazine 2%
- Formalin 10%, ethanol alcohol 96%, xylol, paraffin wax, and Hematoxylin and Eosin (H&E) stain.
- Rabbit polyclonal to Transforming Growth Factor (TGF) beta 3antibody from Abcam company UK (ab15537)
- Detection Kits System, Abcam company England.

3. Experimental Design

Twelve male Newzeland rabbits of 1.5 –2kg weight were used in this study, they were divided into three groups according to 3,7, and10 days healing intervals(4 animals for each group) .All animals were injected by a single dose (150 mg/kg B.W.) intravenously to induced diabetes. After elevation of blood glucose level, the rabbits received subcutaneous injection of insulin as a treatment in a dose of 0.1mg/kg B.W. to control the hyperglycemia (**11**).Then two incisional wound were done on both sides of cheek mucosa of each rabbits, the right incision (experimental group), where filled with mixture of propolis, black seed and honey, the other incision was done at the left side (control group) and left to heal spontaneously. Histological and immunohistochemical evaluation was performed for all healing intervals.

4. Results

Histological findings

Three days duration

Control group: Microphotograph view of wound site of 3days duration shows obvious infiltration of inflammatory cells, blood clot seems to fill wound site, fibroblasts are noticed along with remodeling collagen fibers (Figure1, 2).

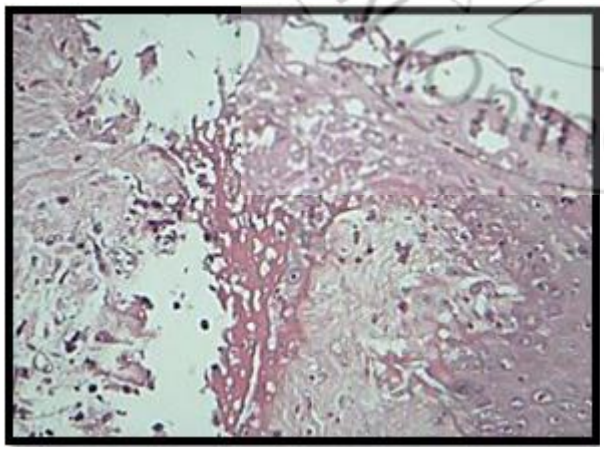

Figure 1: View of 3days duration of control side shows cut edge of wound filled with blood clot and infiltrated by inflammatory cells.H&Ex20.

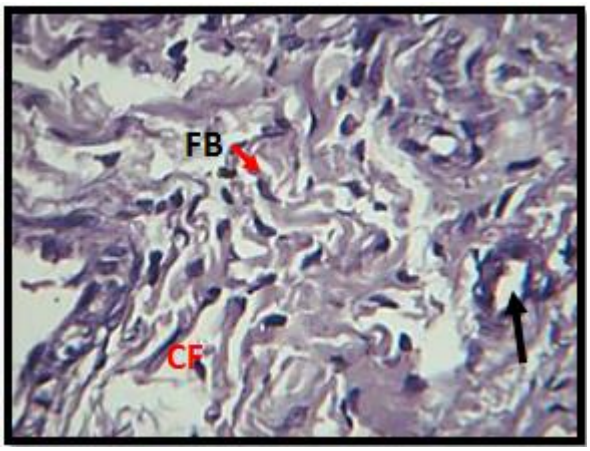

Figure2: View of 3days duration of control side shows remodeling collagen fibers(CF) and formative fibroblasts(FB) and blood vesseles(arrows).H&Ex40.

Experimental group

View of 3 days duration of experimental group shows new epithelium almost sealing wound, the dermis shows organizing collagen fibers and fibroblasts (Figures3,4).

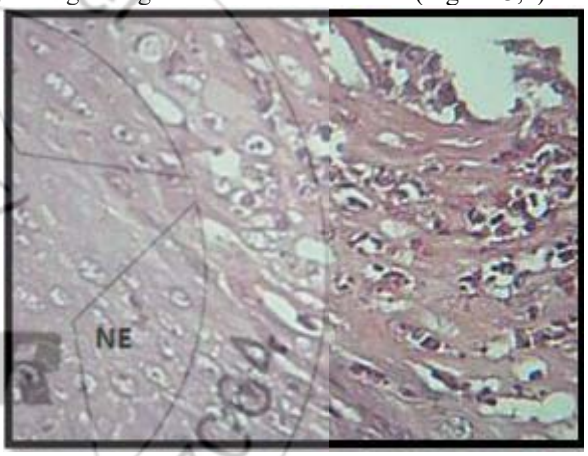

Figure 3: View of 3days duration of experimental side shows new epithelium (NE).H&Ex20.

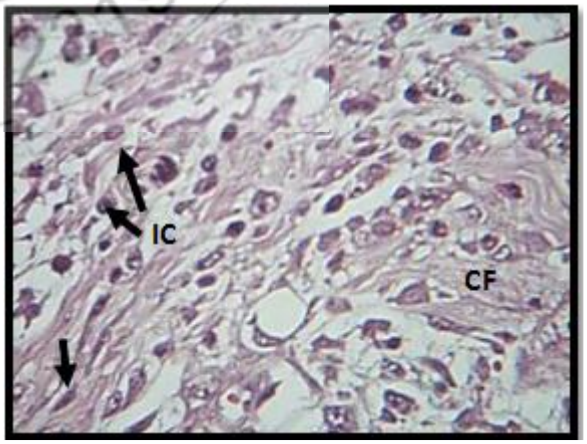

Figure 4: Magnified view of 3days duration of previous figure shows connective tissue infiltrated with inflammatory(IC) cell, new collagen fibers (CF) and formative fibroblasts (arrows).H&Ex40.

Seven days duration

Control group

After7days the histological examination shows newly formed epithelium, fibroblasts and collagen fibers (Figure 5, 6).

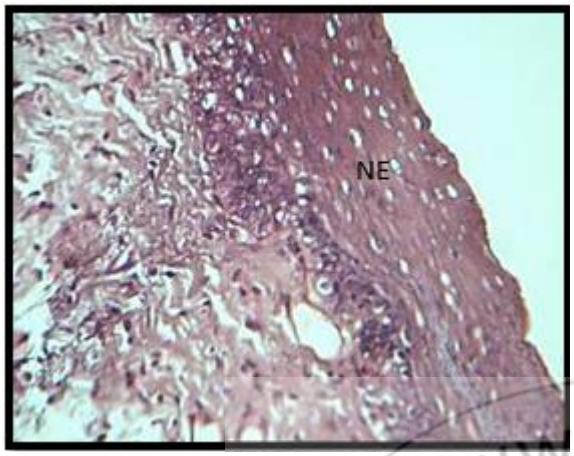

Figure 5: View of 7days duration of control sideshows new epithelium (NE) underlined by fibrous connective tissue. H & Ex20.

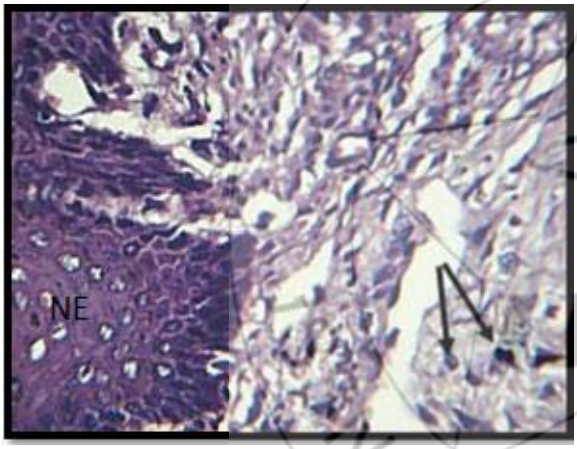

Figure 6: View of 7days duration shows new epithelium (NE) underlined by organized fibers and fibroblasts (arrows).H&Ex40.

Experimental group

Microphotograph view shows wound site which is sealed by epithelium, besides organized connective tissue and, fibroblasts are seen (Figures 7, 8).

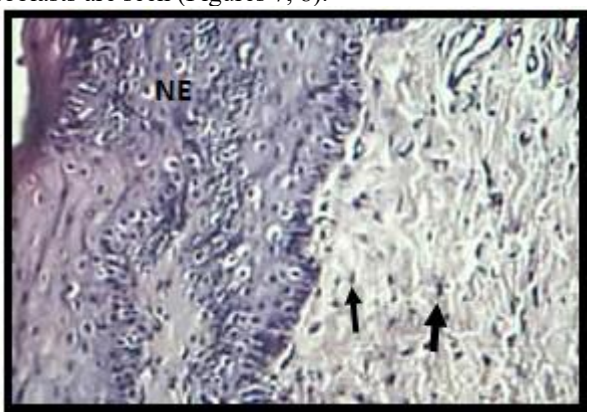

Figure 7: View of 7days duration of experimental side shows new epithelium (NE), and fibroblasts (arrows). H&Ex20.

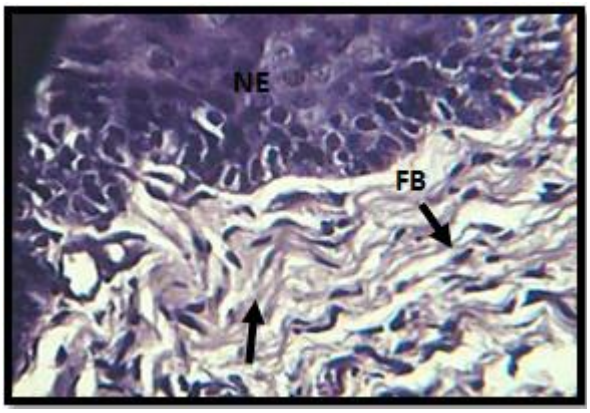

Figure 8: Magnified view of 7days duration shows new epithelium (NE) underlined by organized fibers (arrow) and fibroblasts (FB).H&Ex40.

Ten days duration

Control group

View of 10 days duration shows epithelial cell layers, connective tissue fibers with fibroblasts and blood vessels (Figure 9,10).

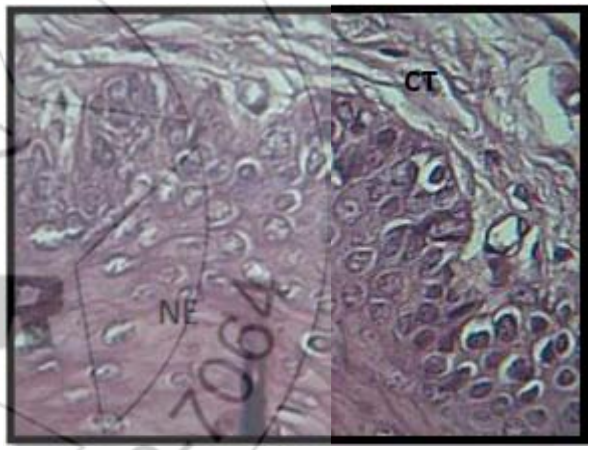

Figure 9: View of 10days duration of control side shows new epithelium (NE) fibrous connective tissue (CT).H&EX40.

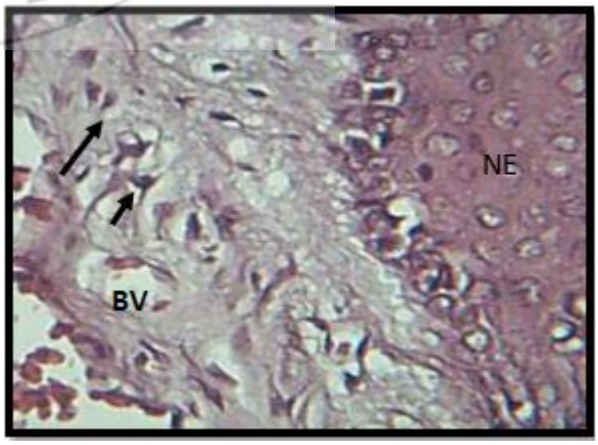

Figure10: View of 10 days duration of control group shows new epithelium (NE) collagen fibers associated with fibroblasts (arrows).H&Ex40.

Experimental group

After 10 days of application of the mixture at wound site, the histological section showed thickened epithelium and well organized connective tissue(Figures11,12).

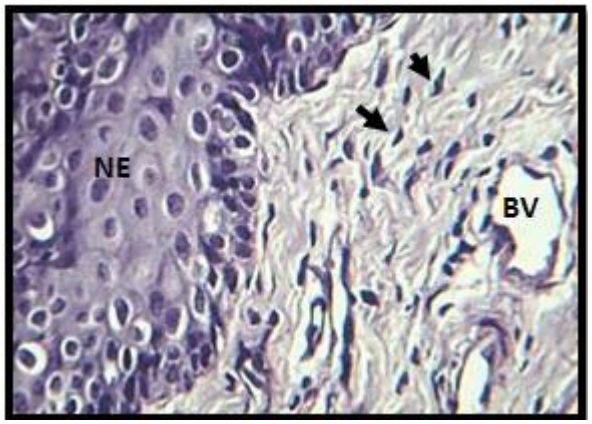

Figure11: View of 10 days duration of experimental side shows new epithelium (NE) collagen fibers and fibroblasts (arrows).H&Ex40.

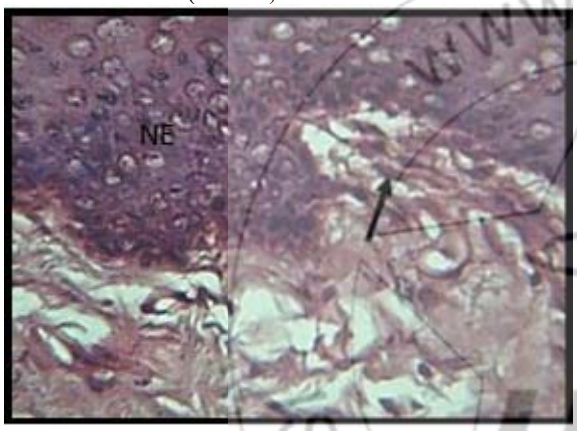

Figure12: View of 10 days duration of experimental side shows thickened new epithelium (NE) collagen fiberss associated with fibroblasts (arrows).H&Ex40.

Immonohitochemical results

Three days duration

Control group

Microphotograph view of 3days duration shows positive localization of TGF-β3 by migrating epithelial cells seen at wound surface, and granulation tissue (Figures13, 14).

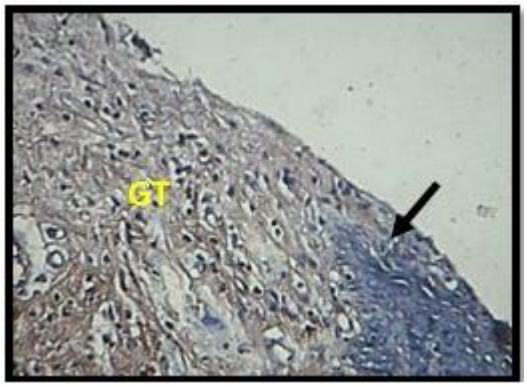

Figure13: Immunohistochemical positive localization of TGF-β3 is detected by migrating epithelial cells seen at wound surface (arrow), and granulation tissue (GT). DAB stain with counter stain hematoxylinX20

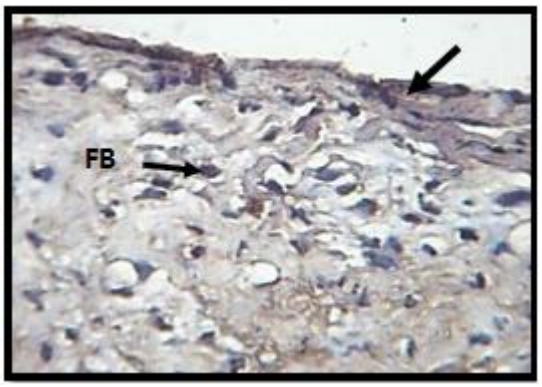

Figure14: Magnified view shows immunohistochemical positive localization of TGF-β3 is by migrating epithelial cells seen at wound surface (arrow), and fibroblasts (FB). DAB stain with counter stain hematoxylinX40

Experimental group

Immunohistochemical localization of TGF-β3 is expressed by progenitor cells, fibrous connective tissue, fibroblasts and endothelial lining of blood vessels as shown in figures15.

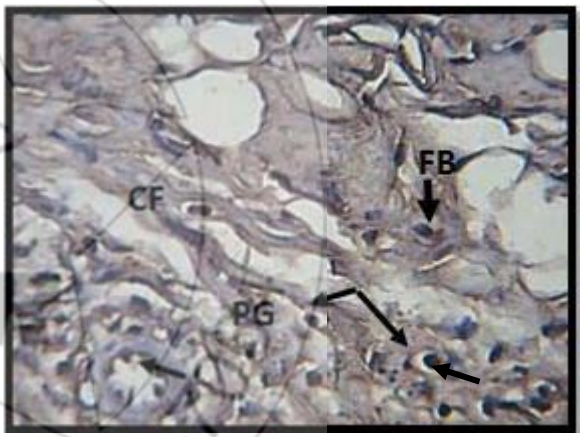

Figure15: View of 3days duration group shows positively stained collagen fibers (CF), and fibroblasts (FB), endothelium (arrows) and progenitor cells (PG).DAB stain with counter stain hematoxylinX40.

Seven days duration

Control group

Microphotograph view after 7 days of control group, shows positive localization of TGF, detected by epitheliumsealing wound surface, connective tissue (Figure 16).

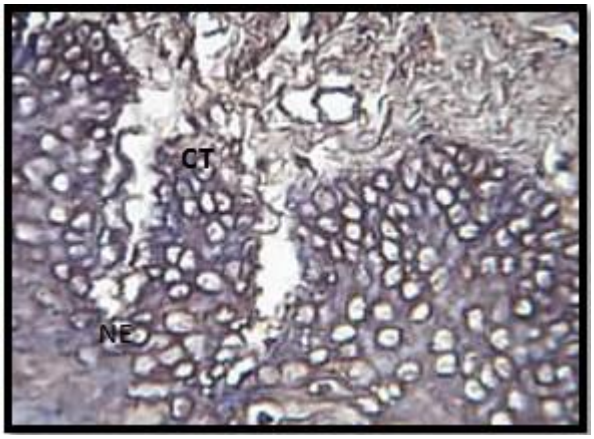

Figure16: View shows positive expression of TGF-β3 by new epithelial cells at wound surface, fibrous connective tissue (CT). DAB stain with counter stain hematoxylin X20.

Experimental group

After 7days the expression of TGF-β3 was detected by epithelium, endothelial lining of blood vessels and fibroblasts (Figure17).

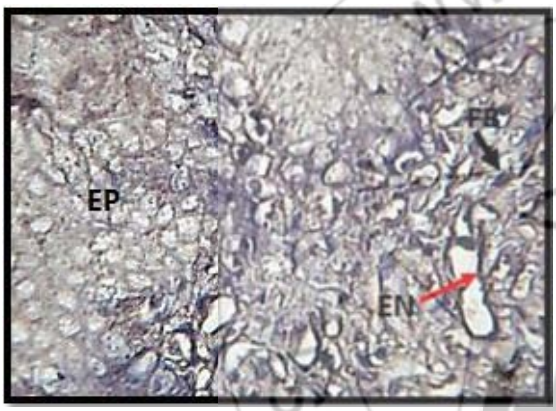

Figure17: View shows positive expression of TGF by epithelial cells at wound surface, vascular endothelium (EN), and fibroblasts (FB). DAB stain with counter stain hematoxylinX40.

Ten days duration

Control group

Positively stained epithelial cells and fibrous connective tissue were detected after 10 days as seen in figure18.

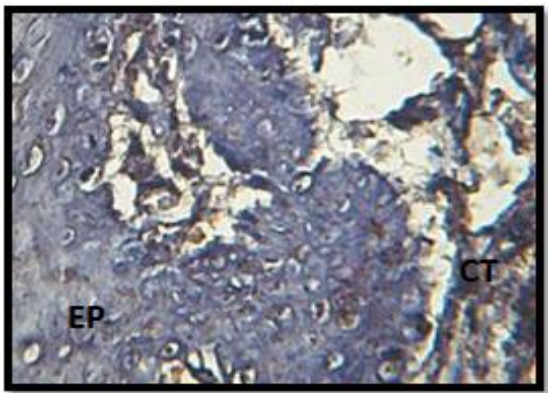

Figure 18: View shows positive expression of TGF-β3 by epithelial cells at wound surface, and connective tissue (CT). DAB stain with counter stain hematoxylinX40.

Experimental group

Immunohistochemical localization of TGF-β3 is detected by positively stained epithelial cells and collagen fibers of dermis (Figures 19).

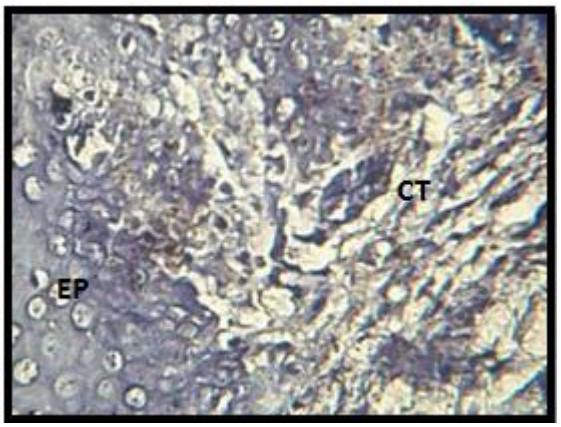

Figure19: View shows positive expression of TGF-β3 by epithelial cells, fibrous connective tissue (CT).DAB stain with counter stain hematoxylinX40.

5. Discussion

Wound healing is a complex process that involves inflammation, granulation and tissue remodeling. Interactions of different cells, extracellular matrix proteins and their receptors are involved in wound healing, and are mediated by cytokines and growth factors **(12).**

The use of herbal therapies for caring of wounds and injuries has been popular since ancient civilizations. In contrast to only 1–3% of modern drugs being used for the treatment of wounds and skin disorders **(13)**

The results of this study showed clear promotion and acceleration of healing process in the experimental groups with mixture of propolis, black seed and honey. The histopathological examination observed that the good response of these groups may be related to stimulation of inflammatory cell or activation of the chemotactic factor, The combination of these materials was probably active to absorb toxins from the mucous membrane and precipitates protein, thus protecting the underlying tissue and enhanced epitheliazation since diabetes was controlled so there was almost no possibility of healing impairment. Atat3days period, the wound site filled with a highly vascularized and proliferating granulation tissue. Also confined by study conducted by **(14),** where histopathological findings showed hemorrhage with inflammatory cell infiltration, as well as congested blood vessels. At 7days ,histological findings showed, thin new epidermis covering wound surface in studied groups, and fibrous connective tissue ,with fibroblasts and remodeling collagen fibers few blood vessels, which was obviously seen in experimental groups where complete reepithelialization at the surface, besides presence of collagen fibers was evident in agreement with **(15)**.At 10 days, reepithelialization was complete and thickened, The underlying dermis showed mature organized collagen fibers, agreed with findings of **Lemo et al., in 2010(14).**

Immunohistochemical evaluation

The repair process is initiated immediately within 24 h of injury by the release of various growth factors and cytokines which initiate the proliferative phase of wound repair and remained high until the repair process was completed. Fibroblast differentiation and function in the later stages of wound healing, which latter starts with the migration and proliferation of keratinocytes at the wound edge(16).From the TGF-β superfamily, TGF-β types 1, 2, and 3 are involved in almost every stage of wound healing. The presence and concentration of these factors as well as other wound-healing promoting factors, such as IGF-1, EGF, PDGF, ILs, and their ratios determine to a great extent the outcome of the wound healing process (17). Transforming growth factor β (TGF-β3) is a multifunctional growth factor with several crucial roles during normal wound healing (18). TGF-β3 regulates wound re-epithelialization and inflammation and promotes connective tissue regeneration. Regarding the immunohistochemical findings in this study, the positive localization of TGF was detected in both epithelialum and dermal connective tissue and was more prominently increased with time as proliferation and differentiation of cells at wound site increased since these cells have a role in expressing this protein and this was accelerated in experimental groups(19).

6. Conclusions

Topical application of mixture of propolis, black seed and honey represents simple and inexpensive model of wound healing enhancement in controlled diabetics.

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