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Gene expression and histological evaluation of the effect of local exogenous application of Morin /*Moringa oleifera* on wound healing in rats

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ABSTRACT

This study aimed to study the effect of local application of Moran and Moringa Oliveira on wound healing in rats, 60 rats of white male rats, and surgical skin (width 2 cm and depth 0.5-0.6 cm) on the skin. From the cheek of the animal and divided into four groups, the control group, Morin group, Moringa group oleifera and Morin (Moringa oleifera) group, animal scratching was performed for healing periods (2,4 and 7 days), histological cells were found inflammatory, Skin, and contracted wound healing in all animal samples for all healing periods. The gene profile was analyzed in biopsies of previously injured skin and treated murine compared with the molecular-treated molybdenum skin by Mygene Bioneer Korea. Upregulation of IL-6, IL-1β1, CYP1B1 and CXCL1 gene expression and downregulation of psoriasin mRNA identified in samples treated topically with Morin.

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INTRODUCTION

Wound healing is a complex process consisting of four steps, heredity, inflammatory reactions, spread and reshaping, all regulated by cytokines and growth factors released by cells in the affected area. The stages are overlapping and linear for acute wounds, while chronic wounds can be found at different stages of the healing process and are not healed in an orderly fashion. Morin (MR), an antioxidant that occurs naturally, was selected. *Moringa oleifera* (Family: Moringaceae, English: Drum Tree, Sanskrit: shrigru) has been a component of the Indian diet for centuries. It is grown almost all over the country and uses its leaves, fruits and vegetables. Almost all plant parts have been used in traditional medicine practices. Foliage and buds are used from plants like vegetables and can be rubbed on temples to relieve a headache while root, bark and scorbutic root are considered and can be used externally as antigens (Makris DP *et al.,* 2002). Juice mixed with honey is used to treat eye diseases (Aruna M and Sivaramakrishnan V M 1990). Plant leaves have been reported for antimicrobial blood pressure (Gilani A H *et al.,* 1994), antioxidants, radiation prevention (Anoop V R *et al.,* 2001), anti-inflammatory and diuretic properties (Yuslianti ER *et al.,* 2014).

MATERIAL AND METHOD

Study design

Skin of 60 healthy males rats (divided to 4 groups each group 15 rats and this groups divided 3 subgroups to each), (Morin and *Moringa oleifera*) every 24 h; group I received no application (3 subgroups 24h, 72h and 144h). group II received Morin applications (3 subgroups 24h, 72h and 144h). Group III received *Moringa oleifera* applications (3 subgroups 24h, 72h and 144h). Group IV received Morin and *Moringa oleifera* together applications (3 subgroups 24h, 72h and 144h). All animals (5 rats per period), we sacrifice mice each period. Samples All groups were stored in 10% formalin for histological analysis, while biopsies were stored in RNAlater (Qiagen, Healden, Germany) at

Table 1: Study deigns

Il-6	F	TGAACAGCGATGATGCACTG	138
	R	AGAAACGGAACTCCAGAAGACC	
Cyp1b1	F	TCAACCGCAACTTCAGCAAC	100bp
	R	ATGAAGGCGTCCATCATGTC	
Cxcl1	F	GCTGGCTTCTGACAACACTAG	148
	R	AACGACCATCGATGAAACGC	
IL-1β1	F	TTCCCACCAACTGTGTTTCC	99bp
	R	TCACACTTGGGTCAACATGG	

-20°C before RNA extraction. The protocol is described by the manufacturer of TRIzol® bags.

THE RESULTS

Morin and *Moringa oleifera* Affect gene expression in the skin in this study, the skin was infected by 60 intact male mice with the blade and subsequently treated skin lesions topically with Morin /*Moringa oleifera* every 24 h. Four groups were received with 3 different application topics: in the group, I did not receive any application, in the second group received morin requests, in the third group received Moringa Oleifera requests and in the fourth group received Morin and Moringa oleifera together applications. Biopsies were taken from all mass treatment areas after the last application after 24 (group I), 72 (group II) and 144 hours (group III) for all periods. Significant effects of Moringa Oliveira on gene regulation can be detected compared to Moran's treatment in all samples from all three-time points analyzed. The effects of Maureen and Moringa Oliveira can be detected together on gene regulation in all samples from all three-time points analyzed. To identify the basic genetic network responsible for the biological effect of the combination (Morin and Moringa oleifera), we have built a basic interactive network of genes that are organized with at least a 2.0-fold change using the Gene Spring Pathway analysis tool. Several genes (IL-6, IL-1β1_, CYP1B1, CXCL1).

Effects of Applications of Morin and *Moringa oleifera* on Gene Expression in Skin

The significant effects of Moringa Oleifera on gene regulation compared with Morin therapy can be detected in all samples of all three-time points analyzed but especially in group IV (Morin and Moringa oleifera applications), we can detect gene increase after Oleifera Moringa treatment by Gene chip analysis. We focused on the expression patterns of highly regulated genes and confirmed these genes by real-time quantitative quantification. Therefore, total RNAs were isolated from punch biopsies of Morin and Moringa oleifera - skin treatment after another of the total applications was subjected to real-time quantitative PCR analysis using specific probes to detect mice IL-6r and IL-1β1_ and CYP1B1 and CXCL1-mRNA. In biopsies of moninga extracted from the skin, high mRNA levels can be observed at the IL-6r level compared with IL-6 mRNA levels detected in biopsies of the Morin processor control skin (Figs. 1, 2 and 3). As shown (Fig. 4, 5 and 6), the treatment of Moringa oleifera excels to alter the fold of the expression IL-1β1_and CYP1B1 (Fig. 7, 7 and 9) compared to the Morin treatment. Skin treatment with Moringa oleifera CXCL1 (Figure 10-11 and 12) Expression after Oleifera Moringa treatment compared to Morin therapy can be shown.

Histological findings

At 24 hours (control groups), after one day of incision, the relative theory of the injury site of the control group shows the defect area, and no epithelium and epithelial cell migration is formed after, microvascular presentation of the wound site in the dermis shows high infiltration of inflammatory cells, Brown *et al.,* 2003; Subhash S and Subramanian P 2009). While Moringa oleifera groups, 24 hours at the wound site of the experimental group show no epithelial region and are epithelial cells are formed after, the presentation of the dermal segment shows the composition of the granular tissue by the proliferation of fibroblasts and the formation of collagen fibres. Moran groups, 24 hours at the wound site of the experimental group show no epithelial area and epithelial hormone cells are formed so far, showing a magnified display of the skin section forming the granulation tissue by fibroblast proliferation. The mixed groups (Morin and Moringa oleifera) for 24 hours at the wound site of the experimental group showing an area of epithelial cells and migration also have a granular tissue formation, a magnified view of the skin section showing the formation of the granular tissue by the proliferation of fibroblasts and formation of collagen fibers and numerous new blood vessels.

In 72 hours (control group), tissue results at the site of the control group lesions for 72 hours show full epithelial mating and there is a decrease in the number of inflammatory cells with the formation of soft collagen fibres, while the other appearance

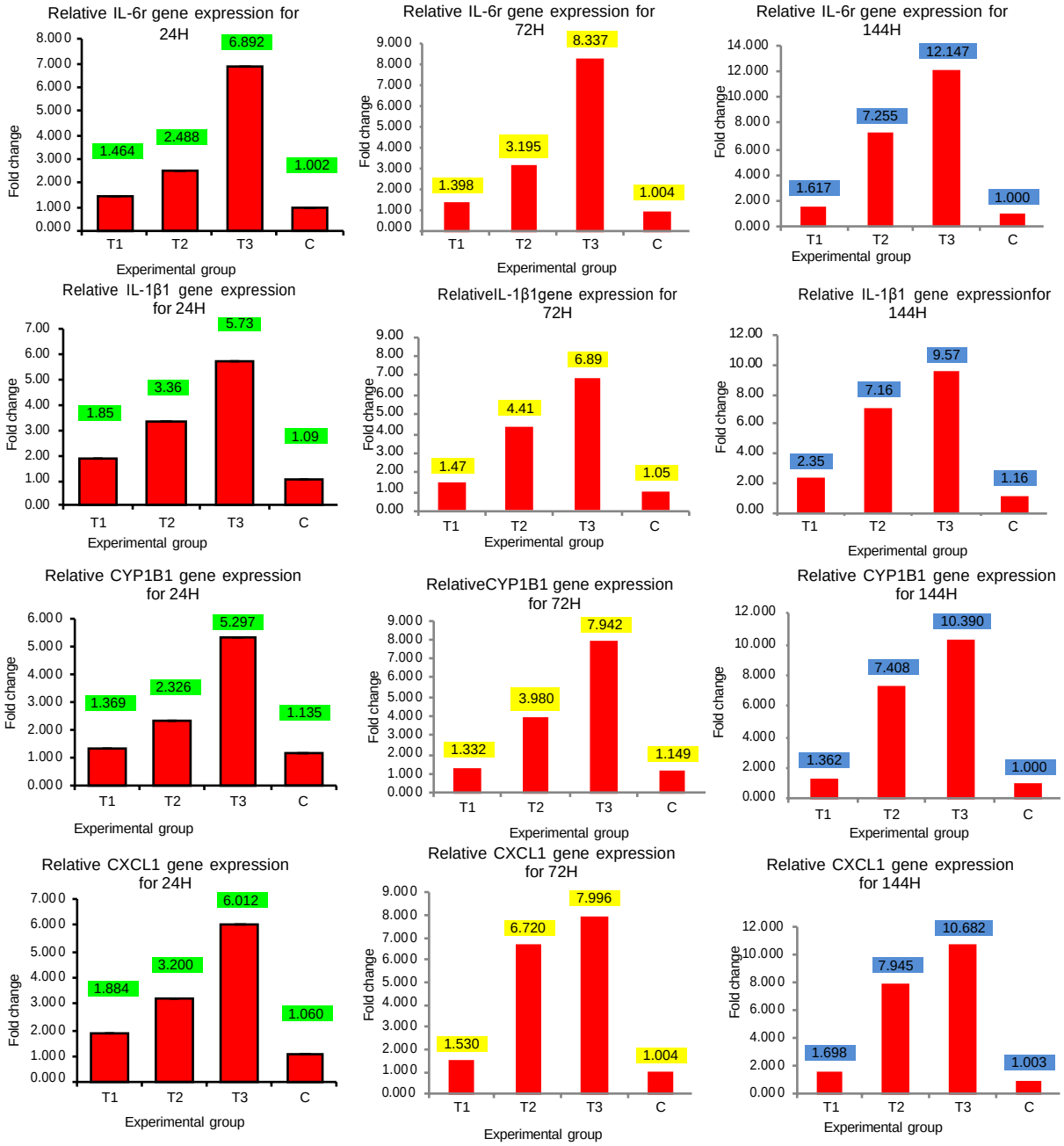

Figure 1: Relative IL-6r, IL1B, CYP1B1 and CXCL1 genes expression for 24, 72 and 144H

shows granulation tissue with irregular collagen arrangement of fibres and properties.

The pathogenesis of endothelial cells, and new blood vessels. Moringa oleifera group showed 72 hours reduction in inflammatory cells and replacement of fibrous tissue by fibres with diffuse fibroblasts, and a complete epithelial layer was observed. The other micrograph shows condensed collagen fibres with signs of re-growth accompanied by active fibres and forms new blood vessels.

Morin group showed a 72-hour reduction in inflammatory cells and replacement of fibrous tissue by fibrous fibrosis with scattered fibroblasts and completely incomplete epithelium is seen in the other microphotograph. And the mixed group (Moran and Moringa oleifera), 72H duration showed the extension of the epidermal layer at both the incision edge and reduction of inflammatory cells and replacement of the fibrous tissue by fibrous fibroblasts with sporadic and complete epithelial tissue formation. The other microscopic image is also seen showing collagen fibres abbreviated with re-markers. The presentation is accompanied by active fibroblasts and the formation of new blood vessels and the proliferation of epidermal cells.

In the 144 hours, the control group shows 144 hours of re-demonstration with a full basebase, along with the restructuring of the collagen fibres, displaying the new vascular formation and active fibroblast with the reconstitution of the collagen fibres to be detected. While the Moringa oleifera group, the skin section at the wound site of the

experimental group reveals a complete epithelial appearance, the new epithelium will be thin with no residue, and the location of the wound that has been cured using the fibrous and reactive cellular component, epithelial layers of the new and little skin Fibroblasts are seen scattered all over the fibrous of denim and forming new blood vessels. Moran Group, Morin group, The Moran group, a 144-hour presentation of the skin section at the wound site of the experimental group that reveals the complete reflection of the epithelium, the new thin epithelium with no lagging edges, the site of the wound that has been cured with fibroblasts, some fibroblasts seen scattered throughout

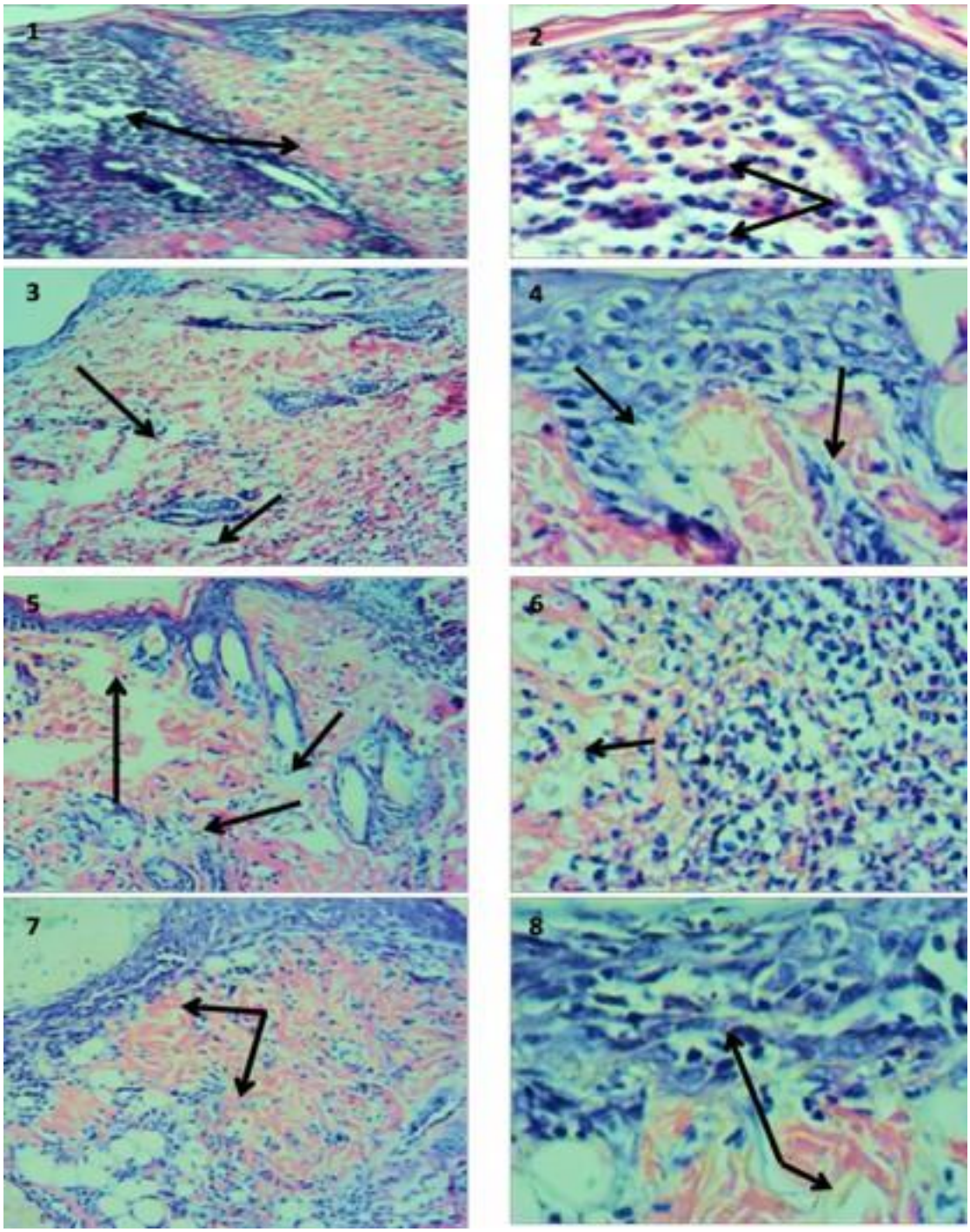

Figure 2: View at the wound site of the control group after 24h shows wound area with, inflammatory cells in the epidermis (arrowhead) H&EX10/40 (Image 1&2); Microphotograph of wound site of 24h morning group duration shows. Granulation tissue at wound area, migrating epithelial cells (arrowhead), collagen fibre (arrow) H&EX10/40 (Image 3&4); Microphotograph of wound site of 24h morin duration shows. granulation tissue at wound area, migrating epithelial cells (arrowhead), collagen fibre (arrow) H&EX10/40 (Image 5&6); View of 24 h duration in combination (morin& moringa) group shows, collagen fibre (arrow), Epithelial cells (arrowhead), Fibroblast (FB) H&EX10/40 (Image 7&8).

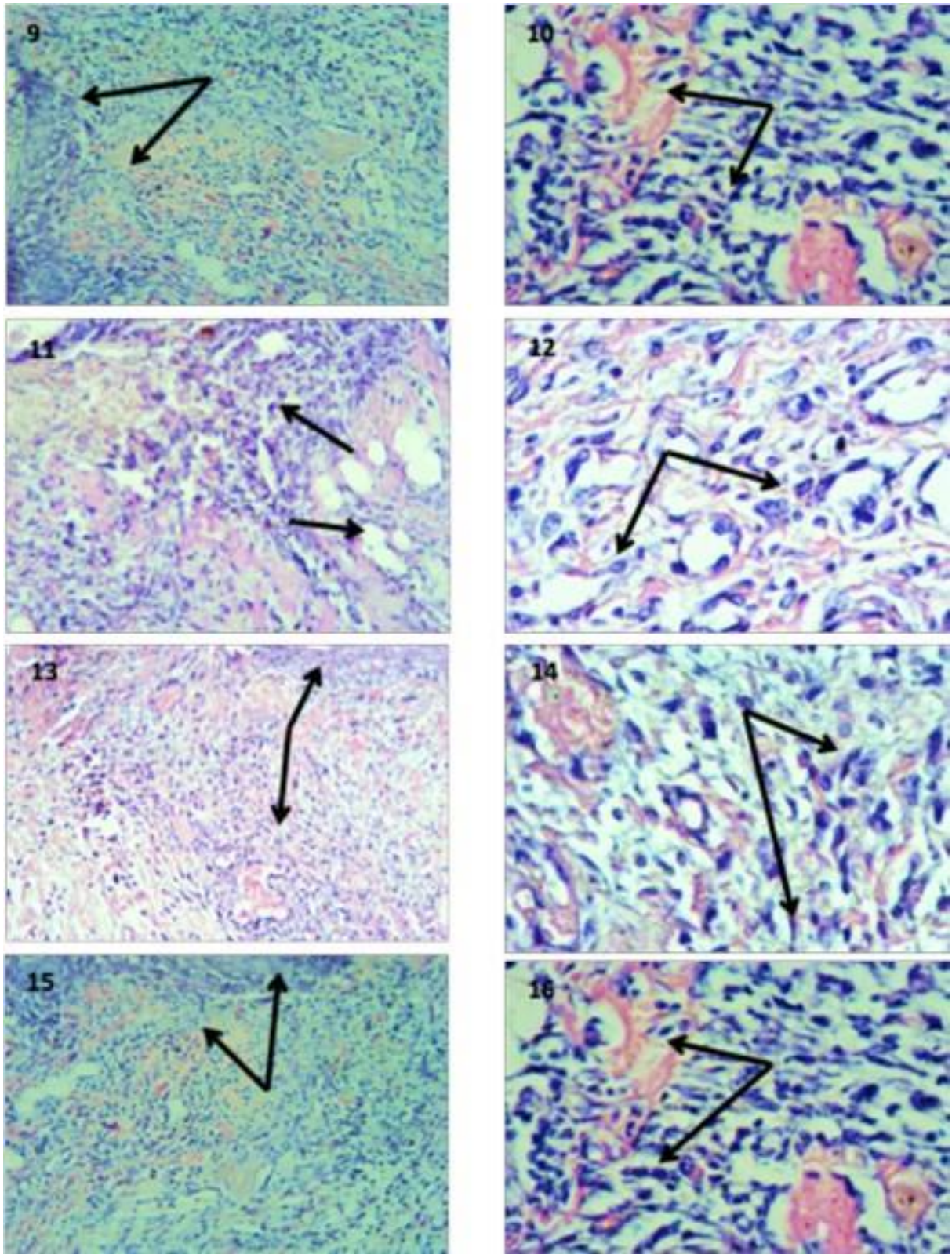

Figure 3: Microphotograph of wound site of 72h duration in the control group shows. Granulation tissue at wound area, migrating epithelial cells (arrowhead), Fat cells, Granulation tissue (GT), Collagen fibre (arrow), new blood vessel (BV) H&EX10/40 (Image 9-10); Microphotograph of wound site of *moringa* group 72h duration shows. Wound area, migrating epithelial cells (head arrow), Fibroblast (FB), Collagen fibre (arrow), new blood vessels (BV) H&EX10/40 (Image 11-12); Microphotograph after 72h of morin application shows. Migrating epithelial cells at wound area, Collagen fibre (arrow) H&EX10/40 (Image 13&14). Microphotograph after 72h of combination group shows. Defect area, complete epithelialization at wound edge, Collagen fibre (arrow), Fibroblast (FB), fat cells (FC) H&EX10/40 (Image 15&16).

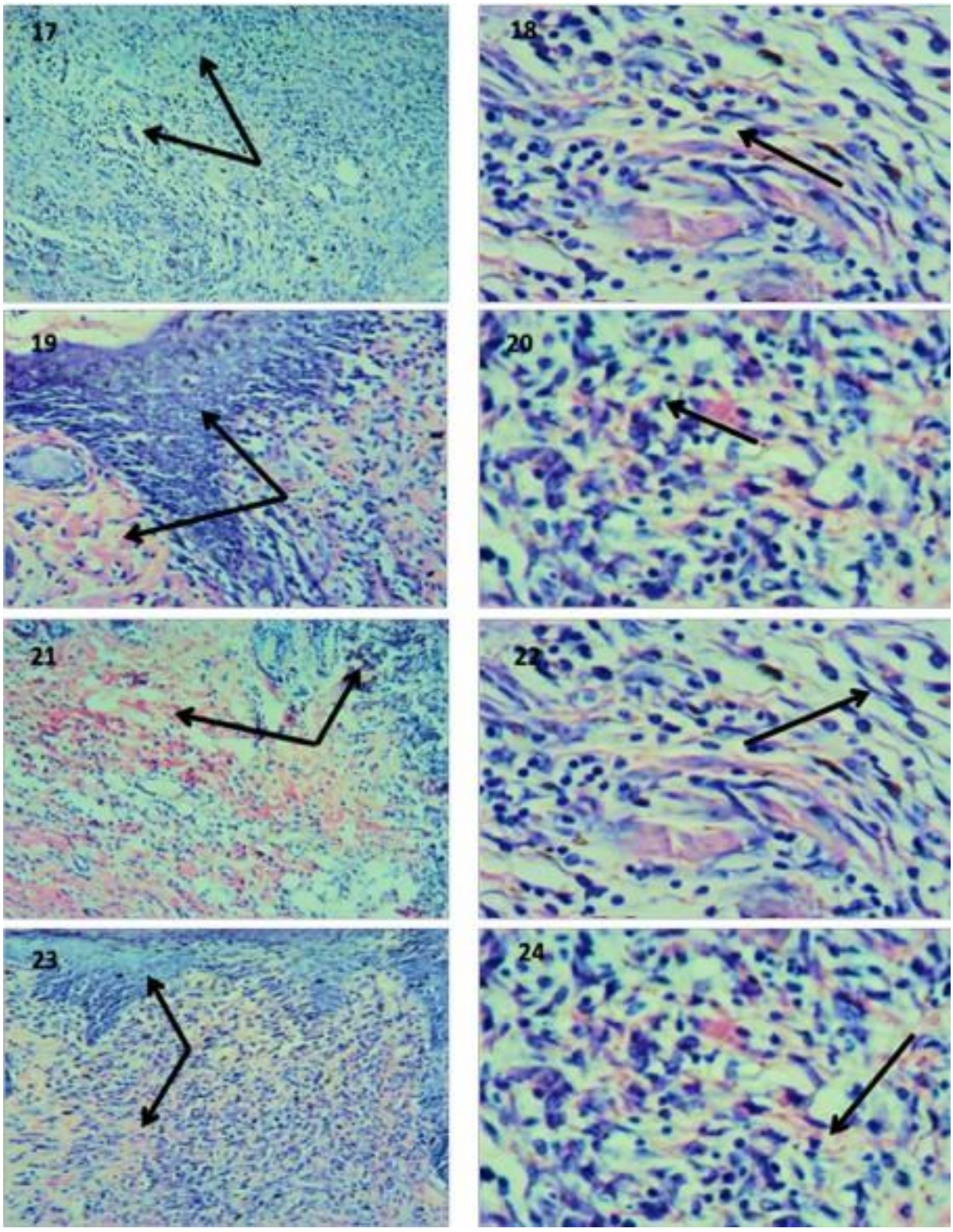

Figure 4: Microphotograph of wound site of Control group 144h duration shows. Complete epithelialization at wound (head arrow). Collagen fibre (arrow), fibroblast (arrowhead) H&EX10/40 (Image 17&18); Microphotograph of wound site of *moringa* group 144h duration shows. Complete epithelialization at wound edge (arrowhead), Fibroblast (FB), new blood vessel (BV) Collagen fibre (arrow) H&E10/40 (Image 19&20); Microphotograph after 144h of morin application shows. Epithelial cells at wound edge, complete epithelialization (arrowhead), Collagen fibre (arrow), Fibroblast (FB) H&EX10/40 (Image 21&22); Microphotograph after 144h of combination group shows. Complete Epithelialization at wound edge, Fat cells (FC), Hair follicles (arrowhead), collagen fibre (arrow). H&EX10/40 (Image 23&24).

the latent lymphocyte formation of the new blood vessels. And the mixed group (Morin and Moringa oleifera), the Microphotograph exhibited at the site of the wound to see the group of the skin section at the wound site was completed by healing the cleft distinguishing it by fusion of both the edge together and revealing the full appearance of the epithelium, the new thin epithelium with no rate can detect the h, The site of the wound healed with fibrous connective tissue with less cellular component, Magnified of other view shows epithelial cell layers of the new epidermis and few fibroblasts are seen scattered throughout the fibrous connective tissue of the underlying denim.

DISCUSSION

The wound can be described as a loss of tissue integrity due to pathological change or physical trauma. Wound healing is a natural process to restore the skin or mucosa to its normal state. The healing of the skin is healed by the same four stages of blood clotting, inflammation, proliferation, and maturation of the matrix/matrix remodelling. Recently, there has been increasing interest in alternative medicines and natural medical products to treat local wounds due to delayed wound healing through traditional pharmacological treatments (Derrick KL *et al.,* 2008). In these studies, the major differences in gene expression can be detected between the three different wound treatments in tissues that were recovered 48 hours after the wound was created. Alteration of gene expression by local topical oliventa treatment for 72 days included regulation of IL-6, IL-1, CYP1B1, and CXCL1 mRNA. It is tempting to speculate that the proliferating fibroblasts are at least partially the source of high ribosome DNA levels in the damaged skin tissue treated with Moringa olivra. However, Prohealing activity can be attributed to its antimicrobial activity, which has been reported earlier. In this study, the wounds were not infected with the naked eye. It is difficult to comment on such activity that contributes to healing. Since systemic administration of zinc sulphate has been reported to promote healing, the prohealing effect of M. oleifera can be observed as observed in the current study because of its high content of zinc as reported earlier. In summary, the extract showed a significant activity to heal wound against abrasion, incision and wound in dead space. Massive claims of medicinal efficacy from various preparations have encouraged the Moringa author and his colleagues at Johns Hopkins University to further investigate some of these possibilities (Morton J P *et al.,* 1972). Moran (3, 5, 7, 2, 4'-penthydro cellaflavone; yellow pigment) is a bioflavonoid component made up of many herbs and fruits. Bioflavonoids is used as herbal medicine and presents many biological activities including antioxidant cytoprotection, anti-anaesthetics and anti-inflammatory. It is reported that Moran can modify the activities of metabolic enzymes, including cytochrome P450, (Hodek P *et al.,* 2002) which is also an antioxidant that protects various human cells, such as muscle cells, endothelial cells, hepatic cells and erythrocytes, against oxidative damage. In addition, the moron acts as a chemical protective agent against oral carcinogenesis in vitro and in vivo. Antioxidant activity of the morin can be attributed to the maintenance of cellular integrity and the body's natural functions are attributed to renoprotective selenium activity. Flavonoids have been used to inhibit the formation of lipid peroxide in mouse tissues and also inhibit the production of free radicals in cells at different stages. Previous treatment with Moran improved SOD and catalase activity by removing superoxide and hydrogen peroxide produced by the International Organization for Standardization (ISO). Moran is a moderately strong inhibitor of Xanthine Oxide (XO). In two separate assays of XO activity, it was shown that morin is more clearly inhibited than this enzyme of Trolox but less than the valorinol. In addition to its ability to "cure" oxidative damage by removing oxydical. The previous report shows that Moran offers protection against hypermagnesemia by reducing oxidative stress and enhanced antioxidant activity in ammonium chloride anaemia (Brown *et al.,* 2003). Flavonoid antioxidants function as scavengers of free radicals by rapid donation of the hydrogen atom to radicals (Subhash, S. and Subramanian, P. 2009; Amy, D *et al.,* 2003), Morin hydrates can effectively protect against oxidative damage in the heart of rabbits during ischemia-infusion through multiple mechanisms. Morin may prevent oxygen generation by inhibition of XO and/or clawing one or more metal ions such as Fe in a cell or organ. The IL-6-deficient (IL-6KO) mice, which exhibited a significant delay in wound healing characterized by reduced epithelial reconstruction, granular tissue and wound closure (Brown *et al.,* 2003) are well known to play an important role in wound healing. In addition, recent studies have shown the secretion of this mediator wound healing of fibrous cells in skin substitutes (Subhash, S. and Subramanian, P. 2009). In contrast, the chemical compound C-X-C ligand 1 (CXCL1) was expressed - signalling through the chemokine CXCR2 receptor - not only in inflammation but also in epithelial cells and fibroblasts. In addition, in vitro lesions, experiments with cultures of keratinocytes formed from CXCR2- / - mice detected wild-type delayed-closure lesions in CXCR2- / - corneal cells, suggesting the role of this receptor on keratinocytes in epithelial emergence which is Independent of the recruitment of

neutrophils (Amy, D *et al.,* 2003). CXCL1 was detected in both the exogenous fibroblast model in the laboratory suggest that fibroblasts are the source of this enhanced mRNA expression. These results suggest that they can analyse aspects of complex processes and can help us determine the individual role of cell types such as the role of fibroblasts in wound healing. But clinical studies indicating skin samples as described in this study remain a prerequisite for understanding the full effects of compounds such as Moringa oleifera on different cell types in rat tissues. In these studies, we can show that treating wounds with Morin and Moringa oleifera once a day completely suppressed recolonization over the test period of 7 days (Amy, D *et al.,* 2003).

CONCLUSION

Morin and Moringa oleifera on gene regulation compared to sham therapy in all samples of all three points of wound healing but especially in those samples that were retrieved after 3 days of the wound. These analyzes, which can be confirmed by qRT-PCR, may provide new insight into the molecular mechanisms responsible for the effect of Morin and Moringa oleifera in wound healing and have shown strong links to previous data in vitro using fibroblasts.

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