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Original Article

Histopathology and Histomorphological Study of Wound Healing Using Clove Extract Nanofibers (Eugenol) Compared to Zinc Oxide Nanofibers on the Skin of Rats

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ABSTRACT

In order to evaluate the healing effect of eugenol and other nanofibers, 100 male Wistar rats (200±10 g) were used with 14-15 weeks of age in this study. All of the male rats were transferred in the standard cages under controlled exposure conditions in a 12:12 h light/dark cycle with a constant temperature about 22±2 ºC. In addition, the male rats were fed with pellets. Firstly, anesthesia process was performed by 2% xylazine hydrochloride (10mg/Kg/IP) and 10% ketamine hydrochloride (100mg/Kg/IP), and then the rats were placed on the operating table. Then the dorsal surfaces of the rats' skin to ileum were scrubbed and prepared as the next step. A circular wound (with a 7 mm diameter) was created by a 7 mm sterile biopsy punch. All 100 rats were divided into four groups (n=25) randomly named as control, nano zinc oxide (ZnO), eugenol nanofibers, and polycaprolactone groups. After that, they were divided into five groups regarding the wound closure rate in days 3, 5, 7, 14, and 21. Then, the wound dressings were placed on the wounds and renewed every 24 h. At the end of days 3, 5, 7, 14, and 21, the relevant tests, such as histopathology, were conducted by removing the tissue volume using a biopsy punch, and then decapitation process was performed on the rats. It was obvious that eugenol nanofiber showed the best granulation tissue by the production of collagen. Further studies are being performed on wound healing by eugenol nanofiber.

Keywords: Nanofibres, Eugenol, Zinc Oxide, Skin, Rat, Wound Healing

Étude Histopathologique et Histomorphologique de la Guérison Des Plaies en Utilisant des Nanofibres d'Extrait de Clou de Girofle (Eugénol) Comparées aux Nanofibres d'Oxyde de Zinc sur la Peau de Rats

Résumé: Dans cette étude, 100 rats Wistar mâles (200 ± 10 g) ont été utilisés à l'âge de 14-15 semaines, afin d'évaluer l'effet cicatrisant de l'eugénol et d'autres nanofibres, Tous les rats ont été transférés dans des cages standards dans des conditions d'exposition contrôlées comprenant un cycle lumière / obscurité de 12:12 et une température constante d'environ 22 ± 2 ºC. De plus, les rats ont été nourris avec des pellets. En premier lieu et juste avant de placer les rats sur la table d'opération, une anesthésie a été effectuée utilisant 2% de chlorhydrate de xylazine (10 mg / kg / IP) et 10% de chlorhydrate de kétamine (100 mg / kg / IP). Ensuite, la surface dorsale de la peau jusqu'à l'iléon des rats a été nettoyée et préparée pour l'étape suivante. Une plaie circulaire (d'un diamètre de 7 mm) a été induite par le biais d'un perforateur de biopsie stérile de 7 mm. Les 100 rats ont été ensuite divisés en quatre groupes (n = 25), considérés de manière aléatoire comme les groupes témoins, nano-oxyde de zinc (ZnO), nanofibres d'eugénol et polycaprolactone. Les rats composant chaque groupe ont été ensuite également distribués dans cinq sous-groupes afin d'évaluer le taux de fermeture des plaies aux jours 3, 5, 7, 14 et 21. Des pansements ont été placés sur les plaies et renouvelés toutes les 24 heures. Au terme des jours 3,

5, 7, 14 et 21, les tests d'histopathologie ont été réalisés en retirant une portion du tissu à l'aide d'un poinçon pour biopsie avant de procéder à la décapitation des rats. Il était évident que la nanofibre d'eugénol présentait le meilleur tissu de granulation grâce à la production de collagène. D'autres études sont en cours sur la cicatrisation des plaies à l'aide de nanofibres d'eugénol.
Mots-clés: Nanofibres, Eugénol, Oxyde de zinc, Peau, Rat, Cicatrisation des plaies

INTRODUCTION

The wound healing process is a complex and organized phenomenon that occurs after skin damage and soft tissues and sometimes lasts for months and years. Certain processes, such as reconstruction, migration, proliferation of parenchymal cells and connective tissue cells, rebuilding the connective tissue, and angiogenesis, are performed during the repair process. There are several stages regarding the wound healing process, including coagulation, inflammation, granulation, fibroplasia, collagen deposition, wound contraction, and epithelisation (Young and Dyson, 1990). Based on pharmacological studies, the accelerated activity of many herbal medicines containing natural materials and compounds (e.g., tannins, terpenoids, and flavonoids) can increase the rate of wound healing due to accidents and certain diseases (Cushnie and Lamb, 2011). The examination on the 3rd or 4th day after the wound creation indicates the inflammatory stage of the wound healing process (Griga et al., 1998), followed by the proliferation process. Therefore, the reduction of inflammation or bruise can speed up wound healing (Diegelmann and Evans, 2004). Performing the tests on the 7th day after the wound creation represent the stages of proliferation and reconstruction of the wound healing process. Activated epidermis cells produce large amounts of vascular endothelial cell growth factor. The basic and mainstream fibroblast growth factor may provide conditions for the construction of new blood vessels in the first 3 days of the wound healing process. Moreover, the new vessel endothelial cell growth factor is important for vascularization during the formation of cicatrix tissue on days 4 to 7. The number of new blood vessels and their diameters, as well as increased blood flow rate, accelerate the wound healing process (Nissen et al., 1998). The benefits of clove oil can be attributed to antibacterial (Hemaiswarya and Doble, 2009), antifungal (Yazdanpanah and Mohamadi, 2014), disinfectant, and antivirus properties (Chaieb et al., 2007). Clove essence contains eugenol, carbophilin, alcohol, benzyl, benzoate, demotyledon, furfural, and ethylene. Eugenol, the main ingredient in clove, has soothing (Nangle et al., 2006) and antiseptic properties that are used in dentistry. Essential oils extracted from cloves, dried flowers, Yugina Carpio Filateta are topically used for relieving pain and accelerating recovery, as well as in the flavoring industry (Kong et al., 2014). The main components are the essential oils of phenylalanine, such as carvacrol, thymol, eugenol, and cinnamaldehyde. The bioactive effect of Eugenia Caryophyllus Filatelia has been studied on microorganisms and parasites, including pathogens and herpes. In addition to antimicrobial, antioxidant, antifungal, and antiviral activities, clove essence has anti-inflammatory and cytotoxic properties (Kurian et al., 2006). This study investigated the chemical composition and biological effects of clove essence, as well as the new results of the isolated and separated multi-resistant antimicrobial activity.

Scaffold in texture.

In tissue engineering, a proper scaffold must be provided in addition to appropriate cellular source. Suitable scaffolds in tissue engineering require some key features, such as using bioresorbable and

biocompatible materials. The appropriate scaffold must be also porous and mechanically stable, allowing the cell to pass and control extracellular signals. Scaffolds are very important for tissue engineering and restorative medicine, and they play important roles in the recovery and reconstruction of tissue. Scaffolds can be biologically or synthetically erosive or non-erosive and dependent or non-dependent. Natural polymers, erodible synthetic polymers, and non-erosive synthetic polymers are tissue engineering biomaterials. Natural polymers are the first type of erosive polymers, which are clinically useful. These polymers have better interactions with cells. Natural polymers include proteins (e.g., silk, collagen, gelatine, fibrinogen, elastin, creatine, actin, and myosin), polysaccharides (e.g., cellulose, amylose, dextran, chitin, and glycosaminoglycan), and polynucleotides (e.g., deoxyribonucleic acid and ribonucleic acid). Scaffolding with proper characteristics, including strength, destruction rate, porosity, microstructure, as well as shape and size, is possible through the preparation of polymer scaffolds. Synthetic polymers are more suitable biomaterials due to their properties, such as porosity, specific time of destruction, and good mechanical properties. These polymers are cheaper than bio-polymers; they can be produced on a large scale with uniform quality and have a longer shelf life. Porous polymer scaffolds with homogeneous pores are very useful in tissue engineering. The proper dimensions of the scaffold pores vary depending on the cell and texture (Yan et al., 2009). Quick amendments are required in order to prevent infection in open skin scar. Skin cross-linking problems are avoided by artificial skin replacement. An ideal material as a powerful scaffold that can prepare well skin tissue regeneration has not been provided yet. Some of collagen or gelatine scaffolds that are currently in use are suitable for the promotion of tissue regeneration; however, they do not have strong mechanical properties. Some other scaffolds made of biodegradable polymers, such as poly (L-lactic acid) (PLLA), are stronger but not so suitable for tissue growth. Recently, the researchers have investigated the use of hybrid scaffolds composed of collagen and synthetic polymers. The connective tissue cells grow in hybrid scaffolds and penetrate into the scaffold pores. In a compound scaffold, a higher number of cells grow in contrast to the scaffolds made of collagen only. The implantation of these hybrid scaffolds behind the rats has shown that the skin defect is completely restored in those 4 weeks after planting. The obtained results of this study also showed that skin regeneration with hybrid scaffolds was healthier than using pure collagen scaffolds, and there is also less deformation in the skin due to the extraordinary power created by PLLA mesh. The ability of a compound scaffold to promote skin restoration in animals while maintaining its mechanical strength has made them promising materials for skin tissue engineering in the future (Prabhakaran et al., 2009). Skin scars can be caused by mechanical trauma, surgery, decreased blood flow rate, burns, and old age. Most skin scars can naturally heal; however, in cases where damage to the skin is irreversible and widespread, the importance of surgery and use of appropriate skin replacements are inevitable to help the recovery and regeneration of the skin. The reconstruction and restoration of dermis require three-dimensional scaffolds to provide elasticity and strength for the epidermis, as well as keratinocytes for the epidermal layer.

MATERIAL AND METHODS

Clove Nanofiber. The clove extract was purchased from Barijessence Company (Iran). Then, nano eugenol was prepared on similar nanofibers with ethyl-alcohol in the School of Advanced Medical Sciences of Tabriz University. A total of 100 male Wistar rats (200±10 g) with 14-15 weeks of age were used in order to evaluate the healing effect of eugenol and other nanofibers.

Study design and animals. This study was conducted according to the guidelines of the Animal Care Review Board of Faculty of Veterinary Medicine

of Islamic Azad University, adhering to the care and use of laboratory animals; in addition, the study was approved by the Ethics Committee of Islamic Azad University. All of the male rats were transferred in standard cages under controlled exposure conditions in a 12;12 h light/dark cycle with a constant temperature about 22±2C^{o} (Phillips, 2008). Before the experiment, the animals were kept in the laboratory to cope with the stress caused due to the unfamiliar environment (Phillips, 2008). The male rats were fed with pellets received from Dast Chin Animal Feed Company (Iran). These pellets contain soybean meal, fish powder, dicalcium phosphate, calcium carbonate, vitamin and mineral supplements, methionine, lysine, multi-enzymes, phytase, antioxidant, growth stimulant, appetite powder, and other supplements (Suckow et al., 2012).

Surgical procedures and grouping. Firstly, after performing anesthesia using 2% xylazine hydrochloride (10 mg/Kg/IP) (Flecknell, 1993) and 10% ketamine hydrochloride (100 mg/Kg/IP) (Wellington et al., 2013), the rats were placed on the operating table. Then, the dorsal surfaces of the rats' skin to ileum were scrubbed and prepared as the next step. A circular wound (with a 7 mm diameter) was created by a 7 mm sterile biopsy punch. The epidermis, dermal, hypoderm, and epithelial layers were completely removed by excisional wounding. After trauma, 100 rats were divided into four groups (n=25) randomly named as control, nano zinc oxide (ZnO), eugenol nanofibers, and polycaprolactone (PCL) groups. After that, they were divided into five groups regarding the wound closure rate in days 3, 5, 7, 14, and 21. Then, the wound dressings were placed on the wounds and renewed every 24 h. At the end of days 3, 5, 7, 14, and 21, the relevant tests, such as histopathology, were conducted by removing the tissue volume using a biopsy punch, and then the rats were euthanized. The wound healing process was studied after the surgery.

Histological assessments. The number of effective cells in wound healing (i.e., neutrophil, macrophage, and fibroblast), new blood vessels, and the volume of collagen deposition were histologically examined in the present study.

Statistical analysis. The collected data were statistically analyzed using one-way analysis of variance in SPSS software (version 22). P-value less than 0.05 was statistically significant.

RESULTS

Findings of skin parameters indices. The average of acute hemorrhage, new blood vessels collagen production, epithelial thickness, as well as leukocyte and edema scores on the 21st day (Figures 2 and 7) showed that eugenol, PCL, ZnO, and control groups have the most scores, respectively.

Findings of histological assessments. As observed, the histological graphs of control, ZnO nanofibers, PCL, and eugenol nanofibers groups indicated some developments in the wound healing process. In the control group, collagen production in the granulation tissue was weak to a great extent, which was coincident with the bar graphs (Figure 9). Figure 10 depicts the histological graph of ZnO group after 21 days. In the aforementioned group, numerous new vessels proved that wound healing improved in comparison to control one. The epithelium (not to be shown) was moderately developed. In addition, these results are in accordance with the bar graphs. The number of vessels in Figure 11 were higher than those in Figure 10 with well-arranged collagen tissue. The epithelial tissue was softly developed in the PCL group. Finally, the histological graph of the eugenol group (Figure 12) depicts a low number of new vessels with the best regularity in the granulation tissue. The epithelium (arrowhead) was well developed.

DISCUSSION

One of the dynamic processes of restoring tissue layers in damaged tissue is wound healing with special complexity. The process of wound contracture occurs throughout the healing process. Fibroblasts move to the wound sites, which causes faster healing and epithelial layer formation. In summary, some processes occur

after wound healing, such as hemostasis, reduction of inflammation, and production of new blood vessels, followed by proliferation of fibroblasts and collagen deposition. Collagen cross-linking and scar maturation are the last processes called remodeling (Singer and Clark, 1999); therefore, histological analysis is performed after 21 days. In the present study, wound healing was determined in 100 male rats. A circular punch-like wound (with a 7 mm diameter) was created on the shoulder of them (Figure 1). After wound creation, all of the animals were divided into four groups, including untreated (i.e., control), ZnO nanofibers, eugenol nanofibers ($C_{10}H_{12}O_2$), and PCL groups with 25 male rats in each group. The animals divided into five groups (n=5) regarding the wound closure rate in days 3, 5, 7, 14, and 21. The animals under the experiment were kept at normal room temperature. After the application of the dressing materials, the rate of wound contraction was dramatically increased by eugenol nanofibers, PCL (i.e., a bioresorbable and biocompatible material (Suganya et al., 2011), and ZnO nanofibers in comparison to that in the control group, respectively.

Table 1. Results of Kruskal Wallis test to compare mean of acute hemorrhage between the measured days

Groups	Day	Average Rating	Test state	df	P-value
Control	3	4.5	14.959	4	0.005
	5	11.70			
	7	14.10			
	14	16.50			
	21	18.20			
Nano zinc oxide	3	8.10	12.328	4	0.015
	5	8.10			
	7	11.70			
	14	17.20			
	21	19.90			
Polycaprolactone	3	5.70	9.974	4	0.041
	5	10.60			
	7	12.70			
	14	15.90			
	21	20.10			
Eugenol	3	7.50	9.974	4	0.041
	5	9.50			
	7	13.40			
	14	15.40			
	21	19.20			

Table 3. Results of Kruskal Wallis test to compare the mean of new blood vessels score between the measured days

Groups	Day	Average Rating	Test state	df	P-value
Control	3	5	14.080	4	0.007
	5	12.20			
	7	12.20			
	14	17			
	21	18.60			
Nano zinc oxide	3	5.70	10.594	4	0.032
	5	10.70			
	7	12.90			
	14	15.10			
	21	20.60			
Polycaprolactone	3	7.40	16.315	4	0.003
	5	9			
	7	11.20			
	14	15.60			
	21	21.80			
Eugenol	3	8	11.431	4	0.022
	5	10			
	7	12			
	14	13.80			
	21	21.20			

This result was based on pathological and histological experiments in the above-mentioned days. In this study, collagen production, new blood vessels, epithelial thickness, edema creation, leukocyte, and acute haemorrhage were determinative markers. All of the

Table 2. Results of Kruskal Wallis test to compare mean of edema score between the measured days

Groups	Day	Average Rating	Test state	df	P-value
Control	3	4.5	14.959	4	0.005
	5	11.70			
	7	14.10			
	14	16.50			
	21	18.20			
Nano zinc oxide	3	5.70	13.493	4	0.009
	5	10.70			
	7	12.90			
	14	15.10			
	21	20.60			
Polycaprolactone	3	5.70	12.915	4	0.012
	5	10.60			
	7	12.70			
	14	15.90			
	21	20.10			
Eugenol	3	7.60	13248	4	0.010
	5	9.70			
	7	11.50			
	14	15.90			
	21	20.30			

bar graphs in the passage indicated that the average of marker scores for eugenol after 21 days were reported as the highest levels among them. This finding revealed some of eugenol effects in the wound healing process.

Table 4. Results of Kruskal Wallis test to compare mean of leukocyte score between the measured days

Groups	Day	Average Rating	Test state	df	P-value
Control	3	4.5	15.377	4	0.004
	5	11.40			
	7	13.70			
	14	16			
	21	19.40			
Nano zinc oxide	3	7.10	12.722	4	0.013
	5	8.80			
	7	12.70			
	14	17.10			
	21	19.30			
Polycaprolactone	3	6.30	16.194	4	0.003
	5	9.50			
	7	11.70			
	14	16.10			
	21	21.40			
Eugenol	3	7.30	12	4	0.017
	5	9.20			
	7	13			
	14	14.90			
	21	20.60			

Table 5. Results of Kruskal Wallis test to compare mean of collagen production score between the measured days

Groups	Day	Average Rating	Test state	df	P-value
Control	3	4.50	14.959	4	0.005
	5	11.70			
	7	14.10			
	14	16.5			
	21	18.20			
Nano zinc oxide	3	5.70	12.946	4	0.012
	5	10.80			
	7	13.10			
	14	15.40			
	21	20			
Polycaprolactone	3	8.60	8.160	4	0.039
	5	10.80			
	7	12.70			
	14	14.10			
	21	18.80			
Eugenol	3	7.40	16.553	4	0.002
	5	9.20			
	7	11			
	14	15.40			
	21	22			

On the other hand, the wound treated with eugenol nanofibers indicated higher level of collagen deposition and epithelial thickness than those in other groups. Collagen is an important factor that supports cellular tissue and is composed of amino acid and hydroxyproline as a biochemical marker for collagen deposition tissue (Nayak and Pereira, 2006).

Table 6. Results of Kruskal Wallis test to compare mean of epithelial thickness score between the measured days

Groups	Day	Average Rating	Test state	df	P-value
Control	3	4.50	15.377	4	0.004
	5	11.10			
	7	13.70			
	14	16			
	21	19.40			
Nano zinc oxide	3	7.70	13.219	4	0.010
	5	9.60			
	7	11.50			
	14	15.90			
	21	20.30			
Polycaprolactone	3	5.70	12.236	4	0.016
	5	10.50			
	7	12.50			
	14	17.60			
	21	18.70			
Eugenol	3	5.80	17.143	4	0.002
	5	9.40			
	7	11.20			
	14	16.60			
	21	22			

Table 7. Results of Kruskal Wallis test to compare mean of scar score between the measured days

Groups	Day	Average Rating	Test state	df	P-value
Control	3	4.50	14.959	4	0.005
	5	11.70			
	7	14.10			
	14	16.50			
	21	18.20			
Nano zinc oxide	3	6.20	13.050	4	0.011
	5	9.70			
	7	13.20			
	14	15.30			
	21	20.60			
Polycaprolactone	3	5	16.283	4	0.003
	5	10.20			
	7	12.30			
	14	16.50			
	21	21			
Eugenol	3	6.40	12.093	4	0.017
	5	10.30			
	7	12.30			
	14	16			
	21	20			

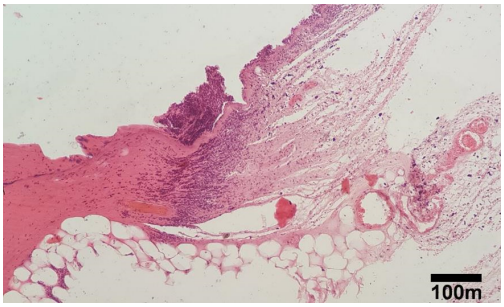

Figure 1. Histological graph of control rat skin after 3 days; note leukocytes infiltration and edema (star) in granulation tissue; the epithelium is not developed (arrow); hematoxylin and eosin staining ×100

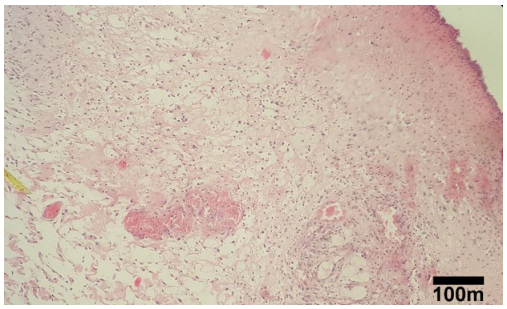

Figure 2. Histological graph of nano zinc oxide treated rat skin after 3 days; note more edema (stars) in granulation tissue; the epithelium is not developed (arrow); hematoxylin and eosin staining ×100

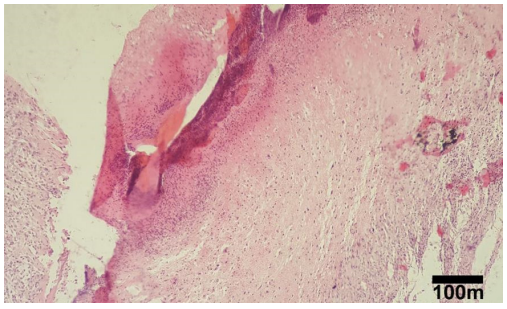

Figure 3. Histological graph of polycaprolactone treated rat skin after 3 days; note leukocytes infiltration (stars) and edema in granulation tissue; the epithelium is not developed (arrow); hematoxylin and eosin staining ×100

For developing the ideal wound healing dressing, the material should have several preferences, such as being nontoxic and permeable for gaseous exchange, resistant to shearing stress, as well as not antigenic and antibacterial. In addition, they should be elastic and flexible with some essential properties, such as the reduction of pain and healing time (Charernsriwilaiwat et al., 2012); therefore, some important markers were chosen in the quest for the investigation of these indices.

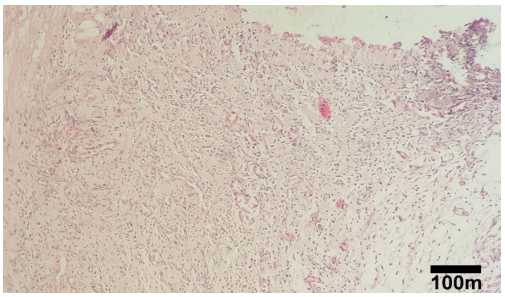

Figure 4. Histological graph of Eugenol treated rat skin after 3 days; note leukocytes infiltration (stars) and edema in granulation tissue; the epithelium is not developed (arrow); hematoxylin and eosin staining ×100

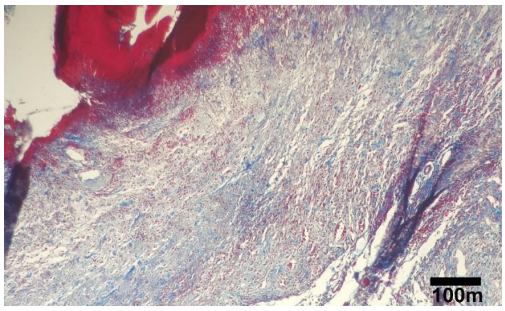

Figure 5. Histological graph of control treated rat skin after 5 days; more edema (star) with moderate collagen production in granulation tissue; the epithelium is not developed (arrowhead); Masson's trichrome staining ×100

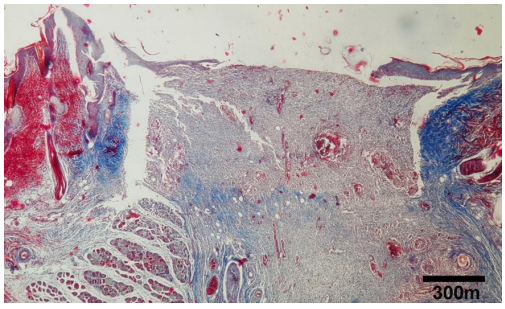

Figure 6. Histological graph of nano zinc oxide rat skin after 5 days; edema (star) and fewer leukocytes infiltration with moderate collagen production in granulation tissue; the epithelium is developed from ulcer sides (arrowhead); Masson's trichrome staining ×100

Alam et al. (2016) examined the effects of clove oil nano-emulsion on the ulcer.

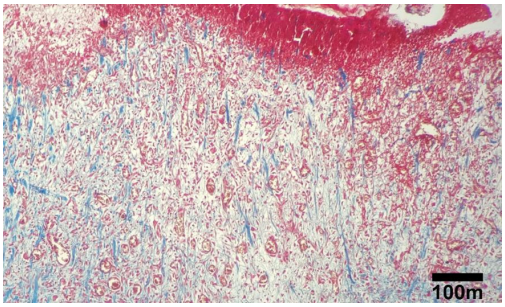

Figure 7. Histological graph of polycaprolactone treated rat skin after 5 days; note edema (star) and numerous new vessels (arrows) in granulation tissue; the epithelium is not developed (arrowhead); Masson's trichrome staining ×100

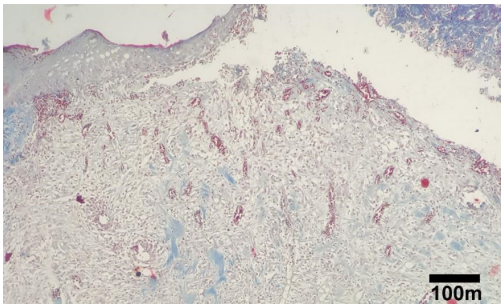

Figure 8. Histological graph of Eunogel treated rat skin after 5 days; note edema (stars) and numerous new vessels (arrows) in granulation tissue; the epithelium (arrowhead) is well developed more than others; Masson's trichrome staining ×100

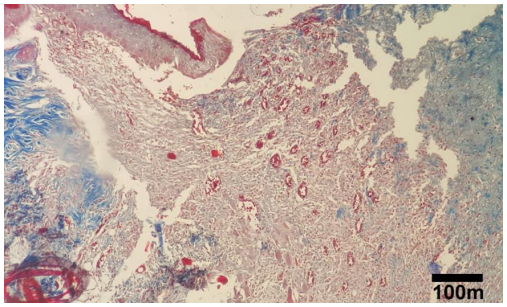

Figure 9. Histological graph of control treated rat skin after 7 days; moderate edema (star) and numerous new vessels (arrows) with low collagen production in granulation tissue; the epithelium is trying to develop (arrowhead); Masson's trichrome staining ×100

The results showed that there is no sign of inflammation. As a result, this material is effective and nontoxic for wound healing (Alam et al., 2016).

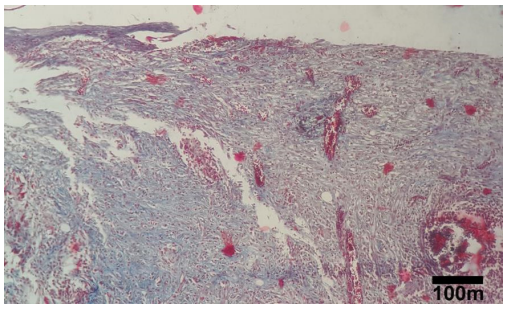

Figure 10. Histological graph of nano zinc oxide treated rat skin after 7 days; note low edema (star) and numerous new vessels (arrows) with moderate collagen production in granulation tissue; the epithelium (arrowhead) is trying to develop; Masson's trichrome staining ×100

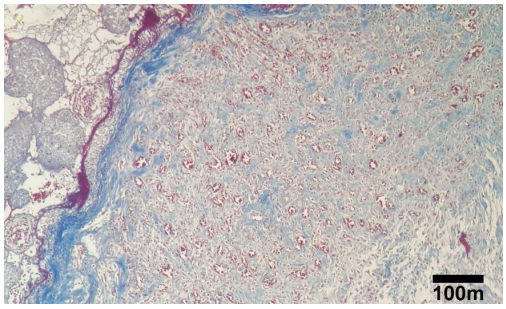

Figure 11. Histological graph of polycaprolactone treated rat skin after 7 days; note severe edema (star) and number of new vessels (arrows) in granulation tissue; the epithelium is not developed (arrowhead); Masson's trichrome staining ×100

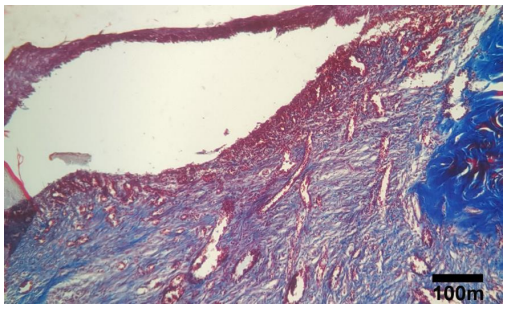

Figure 12. Histological graph of Eugenol treated rat skin after 7 days; low edema (star) and numerous new vessels (arrows) with best collagen production in granulation tissue; the epithelium is developed on ulcer surface completely (arrowhead); Masson's trichrome staining ×100

In a study conducted by Taher et al. (2015), anti-inflammation and anti-bacterial effects of clove extract on the mice skin were evaluated and significant efficacy of the treatment was figured out in contrast to

that of the control group. Briozzo et al. (1989) examined the antimicrobial effects of clove oil on bacteria.

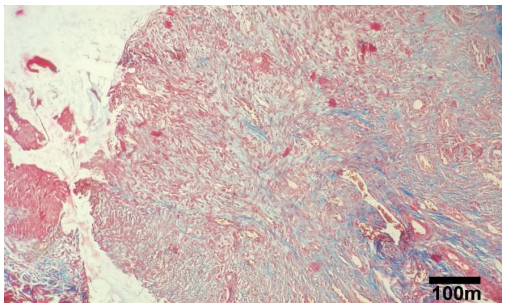

Figure 13. Histological graph of control treated rat skin after 14 days; note numerous new vessels (arrows) and low collagen production in granulation tissue; the epithelium (not to be shown) is moderately developed; Masson's trichrome staining ×100

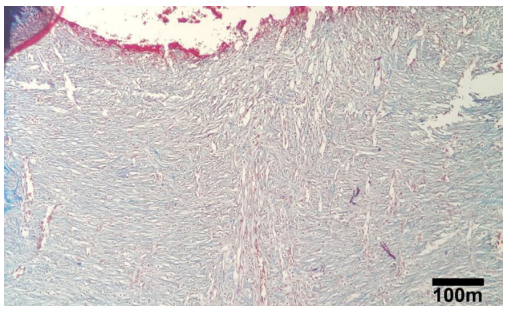

Figure 14. Histological graph of nano zinc oxide treated rat skin after 14 days; note numerous new vessels (arrows) and low well-arranged collagen fibers in granulation tissue; the epithelium (not to be shown) is moderately developed; Masson's trichrome staining ×100

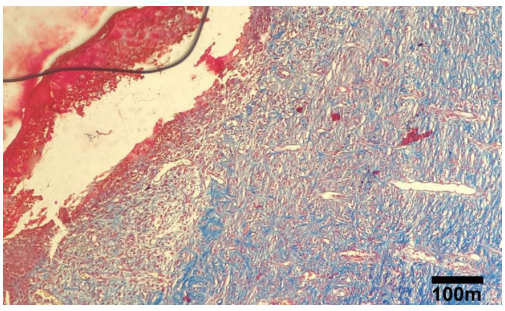

Figure 15. Histological graph of polycaprolactone treated rat skin after 14 days; note numerous new vessels (arrows) and moderate collagen production in granulation tissue; the epithelium (arrowhead) is hyperplastic; Masson's trichrome staining ×100

The results of the aforementioned study indicated that this oil had bactericidal effects on microbial agents and sugar solution. In addition, sugar solution only had a steady role. Núñez et al. (2001) worked on the anti-fungal effects of clove, which were focused with sugar on fungus agents in the present study. The positive results of carnations were demonstrated similar to those of povidone-iodine and chloroxylenol.

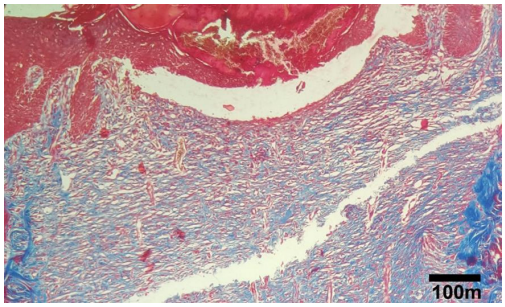

Figure 16. Histological graph of Eugenol treated skin after 14 days; note numerous new vessels (arrows) and high collagen production well arranged in granulation tissue; the epithelium (arrowhead) is well developed; Masson's trichrome staining ×100

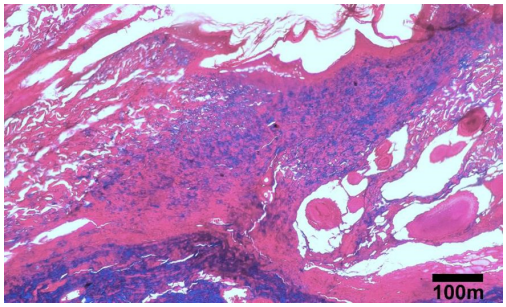

Figure 17. Histological graph of control treated rat skin after 21 days; note moderate collagen production (arrows) in granulation tissue; the epithelium (arrowhead) is moderately developed; Masson's trichrome staining ×100

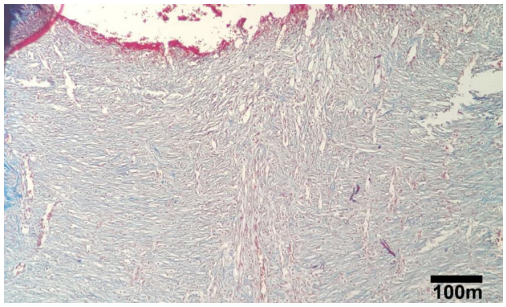

Figure 18. Histological graph of nano zinc oxide treated rat skin after 21 days; note numerous vessels (arrows) and moderate collagen production in granulation tissue; the epithelium (not to be shown) is moderately developed; Masson's trichrome staining ×100

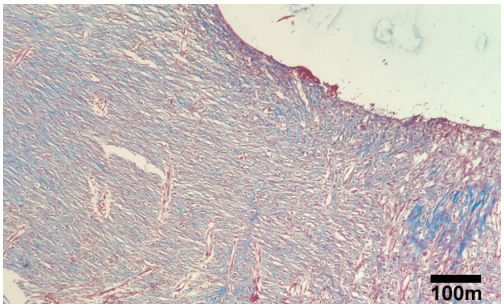

Figure 19. Histological graph of polycaprolactone treated rat skin after 21 days; note numerous vessels (arrows) and well-arranged collagen in granulation tissue; the epithelium (not to be shown) is moderately developed; Masson's trichrome staining ×100

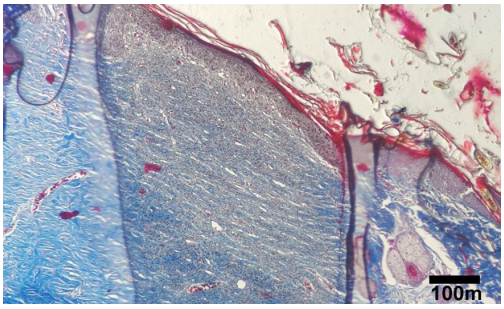

Figure 20. Histological graph of Eugenol treated skin after 21 days; note low vessels (arrows) and well collagen production arranged with the best regularity in granulation tissue; the epithelium (arrowhead) is well developed; Masson's trichrome staining ×100

A wound by the excisional wounding method was created on the shoulder of the rat. Eugenol nanofibers, PCL, ZnO nanofibers have been chosen as wound healing dressings. As illustrated in figures, the healing time by eugenol nanofibers was lower than those of other groups. Several markers, such as collagen production, new blood vessels, epithelial thickness, edema creation, leukocyte, and acute hemorrhage, were determinative ones. The statistical software, such as SAS and MSTATC, were used to determine the cellular count. It was obvious that eugenol nanofibers showed the best granulation tissue by collagen production. Further studies are being performed on wound healing using eugenol nanofibers.

Ethics

We hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

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References

Alam, P., Ansari, M.J., Anwer, M.K., Raish, M., Kamal, Y.K.T., Shakeel, F., 2016. Wound healing effects of nanoemulsion containing clove essential oil. Artif Cells Nanomed Biotechnol 45, 591-597.

Briozzo, J., Nunez, L., Chirife, J., Herszage, L., D'Aquino, M., 1989. Antimicrobial activity of clove oil dispersed in a concentrated sugar solution. J Appl Bacteriol 66, 69-75.

Chaieb, K., Zmantar, T., Ksouri, R., Hajlaoui, H., Mahdouani, K., Abdelly, C., *et al.*, 2007. Antioxidant properties of the essential oil of Eugenia caryophyllata and its antifungal activity against a large number of clinical Candida species. Mycoses 50, 403-406.

Charernsriwilaiwat, N., Opanasopit, P., Rojanarata, T., Ngawhirunpat, T., 2012. Lysozyme-loaded, electrospun chitosan-based nanofiber mats for wound healing. Int J Pharm 427, 379-384.

Cushnie, T.P., Lamb, A.J., 2011. Recent advances in understanding the antibacterial properties of flavonoids. Int J Antimicrob Agents 38, 99-107.

Diegelmann, R.F., Evans, M.C., 2004. Wound healing: an overview of acute, fibrotic and delayed healing. Front Biosci 9, 283-289.

Flecknell, P.A., 1993. Anaesthesia of animals for biomedical research. Br J Anaesth 71, 885-894.

Griga, T., Tromm, A., Spranger, J., May, B., 1998. Increased serum levels of vascular endothelial growth factor in patients with inflammatory bowel disease. Scand J Gastroenterol 33, 504-508.

Hemaiswarya, S., Doble, M., 2009. Synergistic interaction of eugenol with antibiotics against Gram negative bacteria. Phytomedicine 16, 997-1005.

Kong, X., Liu, X., Li, J., Yang, Y., 2014. Advances in pharmacological research of eugenol. Curr Opin Complement Alternat Med 1, 8-11.

Kurian, R., Arulmozhi, D., Veeranjaneyulu, A., Bodhankar, S., 2006. Effect of eugenol on animal models of nociception. Indian J Pharmacol 38, 341.

Nangle, M.R., Gibson, T.M., Cotter, M.A., Cameron, N.E., 2006. Effects of eugenol on nerve and vascular dysfunction in streptozotocin-diabetic rats. Planta Med 72, 494-500.

Nayak, B., Pinto Pereira L.M., 2006. Catharanthus roseus flower extract has wound-healing activity in Sprague Dawley rats. BMC Complem Altern M 6, 41.

Nissen, N.N., Polverini, P.J., Koch, A.E., Volin, M.V., Gamelli, R.L., DiPietro, L.A., 1998. Vascular endothelial growth factor mediates angiogenic activity during the proliferative phase of wound healing. Am J Pathol 152, 1445-1452.

Núñez, L., D'Aquino, M., Chirife, J., 2001. Antifungal properties of clove oil (Eugenia caryophylata) in sugar solution. Braz J Microbiol 32, 123-126.

Phillips, C., 2008. The welfare of animals: the silent majority. Springer Science & Business Media.

Prabhakaran, M.P., Venugopal, J.R., Ramakrishna, S., 2009. Mesenchymal stem cell differentiation to neuronal cells on electrospun nanofibrous substrates for nerve tissue engineering. Biomaterials 30, 4996-5003.

Singer, A.J., Clark, R.A., 1999. Cutaneous wound healing. N Engl J Med 341, 738-746.

Suckow, M.A., Stevens, K.A., Wilson, R.P., 2012. The laboratory rabbit, guinea pig, hamster, and other rodents. First ed. Academic Press, London.

Suganya, S., Senthil Ram, T., Lakshmi, B., Giridev, V., 2011. Herbal drug incorporated antibacterial nanofibrous mat fabricated by electrospinning: an excellent matrix for wound dressings. J Appl Polym Sci 121, 2893-2899.

Taher, Y.A., Samud, A.M., El-Taher, F.E., ben-Hussin, G., Elmezogi, J.S., Al-Mehdawi, B.F., *et al.*, 2015. Experimental evaluation of anti-inflammatory, antinociceptive and antipyretic activities of clove oil in mice. Libyan J Med 10, 28685.

Wellington, D., Mikaelian, I., Singer, L., 2013. Comparison of ketamine–xylazine and ketamine–dexmedetomidine anesthesia and intraperitoneal tolerance in rats. J Am Assoc Lab Anim 52, 481-487.

Yan, S., Xiaoqiang, L., Lianjiang, T., Chen, H., Xiumei, M., 2009. Poly (l-lactide-co-ε-caprolactone) electrospun nanofibers for encapsulating and sustained releasing proteins. Polymer 50, 4212-4219.

Yazdanpanah, L., Mohamadi, N., 2014. Antifungal activity of clove essential oil from Syzygium aromaticum on Paecilomyces variotii agent of pistachio dieback. J Biodivers Environ Sci 4, 42-45.

Young, S.R., Dyson, M., 1990. Effect of therapeutic ultrasound on the healing of full-thickness excised skin lesions. Ultrasonics 28, 175-180.