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The Effect of Aqueous Extract of Robusta Coffee Compared to Neomycin-Bacitracin on Wound Healing by Measuring TNF-1 and bFGF in Fibroblast Cell Cultures

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Abstract.
Coffee grounds have been used to dress wounds for more than 100 years, with excellent results. The wound heals with an acceptable scar. This study aims to compare coffee extract to neomycin-bacitracin in cell migration. Compared to the antibiotic neomycin-bacitracin powder group and control group in scar tissue formation, the Robusta coffee powder extract measured positively on Transforming Growth Factor and Basic Fibroblast Growth Factor markers. This study emphasizes the ability of coffee to heal wounds without causing excessive scarring. Coffee is proven to have better wound healing capabilities than Neomycin-Bacitracin.

Keywords: coffee grounds, fibroblast cell-line, Transforming Growth Factor, basic Fibroblast Growth Factor

1. INTRODUCTION

Wound treatment using home-made coffee powder has been carried out in Indonesia. It is local wisdom [1,2]. Research on coffee for wounds has been carried out since 2003 [1-3] Neomycin-Bacitracin powder is a kind of antibiotic that use for formal topical wound dressing.

This research will answer coffee powder on wound healing that does not cause excessive scarrings such as hypertrophic scars or even keloids. Excessive scar tissue is indicated by examining the levels of markers TGF-β1 (transforming growth factor) and bFGF (Basic Fibroblast Growth Factor) on fibroblast cell cultures in the laboratory [4].

This study determines the quality of scar tissue growth; it was carried out by checking the cytokines TGF-β1 and bFGF.

OPEN ACCESS

2. METHODS

The research was carried out in the cell-lines laboratory at the Aretha Laboratory on Jalan Kebon Jeruk II no.9 Bandung, where Fibroblast cells (CRL-2522) were cultured. Fibroblasts purchased from ABM (Applied Biological Materials, Vancouver, Canada) Immortalized Human Fibroblast, Cat. No: T0345, Quantity ≥1x106 cells / 1.0ml.

3. Coffee

3.1. Material:

1. Human fibroblast cell culture; skin / foreskin (BJ) (ATCC®, CRL-2522)
2. Medium BJ
3. DMSO 100% and 10% (Merck, 1029521000)
4. Coffee powder extract liquid samples (EKL) (PT.Indesso, 17000000 - 0025)
5. Arabica coffee powder extract (EKP) sample (PT.Indesso, TP 757111)

Robusta coffee water extract has been made (coffee extract water = EKL) by PT. Indesso (Cileungsi, West Java) compared to Robusta coffee powder in nano size (nano coffee = EKP) made by PT. Indesso. Dissolved with DMSO (dimethyl sulfoxide, made by Merck, 1029521000).

1. Arabica coffee extract (EKP).

3.2. Coffee Extract Concentration:

Working solution: 5000; 2500; 1250; 622.5; 312.5; 156.25; 78,125 (µg / mL)

Final concentration: 500; 250; 125; 62.25; 31.25; 15,625; 7.8125 (µg / mL)

1. 2) Coffee extract (liquid) (EKL).

Working solution: 5; 2.5; 1.25; 0.6225; 0.3125; 0.15625; 0.078125%

Final concentration: 0.5; 0.25; 0.125; 0.0623; 0.0313; 0.0156; 0.0078%

3.3. Neomycin-Bacitracin (NB):

Neomycin-Bacitracin (Nebacetin) was purchased from the Pharos Indonesia Pharmaceutical Industry, in 5g packs.

Procedure:

Cells were grown at a density of 2x105 cells / well on 24 well plates, then incubated for 24 hours at 37°C, 5% CO2. After 24 hours of incubation, cells were observed under an inverted microscope to determine the level of cell density (about 70-80%). Scratch wound by making a straight line in the middle of the well using blue tips (1 ml). The culture medium was collected and replaced with a new culture medium (with the addition of each with a certain concentration of NB) as much as 500 µl. Cells were then incubated at 37°C, 5% CO2. Observation of scar tissue formation was carried out at 0 and 24 hours after adding the extract.

The culture medium at 24 and 72 hours were collected, then replaced with a new culture medium. The percentage of BJ cell migration is determined using ImageJ application.

Mapping research on the effect of NB on scar tissue formation. Control: BJ cells without the addition of NB.

K-FBS: Non-FBS control

NB 500: Neomycin-Bacitracin 500 µg / mL NB 100: Neomycin-Bacitracin 100 µg / mL NB 10: Neomycin-Bacitracin 10 µg / mL

3.4. ELISA (Enzyme-link Immunosorbent Assay)

1. Conditioned Medium cell BJ (CM-BJ)

3.4.1. Material:

1. Human bFGF / FGF2 ELISA kit (Elabscience, E-EL-H0483)

2. Human TGF-β1 ELISA kit (Elabscience, E-EL-H0110)

4. RESULTS

TABLE 1: Migration of cell data.

Sample		Hour-0		Hour-24
	Area	Migration (%)	Area	Migration (%)
Control	679535	0.00	375179	44.79
Control non FBS	506994	0.00	532041	-4.94
EKP100	528280	0.00	192745	63.51
EKP50	643881	0.00	346949	46.12
EKP10	547893	0.00	305357	44.27
EKL0.1	581006	0.00	429573	26.06
EKL0.05	568898	0.00	252589	55.60
EKL0.01	627941	0.00	356443	43.24

Note: EKP = Arabica coffee extract (ground coffee). EKL = coffee extract (liquid).

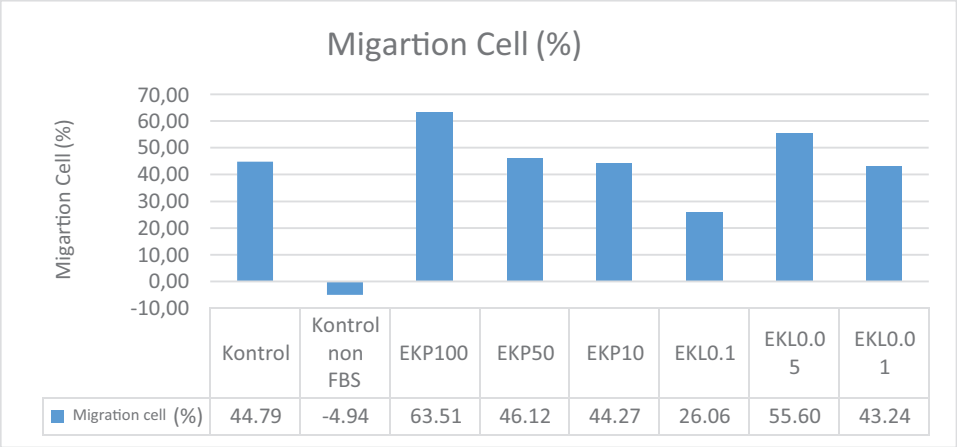

Figure 1: Effect of coffee extract on scar tissue formation.

4.1. Coffee

Note: EKP = Arabica coffee extract (ground coffee). EKL = coffee extract (liquid).

TABLE 2: TGF-β1 concentration.

Sample	TGF-β1 concentration	
	hour-24 (day-1)	hour-72(day-3)
Control	594.40 ± 10.58^{c}	757.32 ± 6.43^{ef}
K-FBS	53.88 ± 6.67^{a}	48.51 ± 8.68^{a}
EKP100	827.18 ± 10.56^{f}	1293.96 ± 48.44^{h}
EKP50	662.96 ± 6.80^{cde}	1013.68 ± 40.01^{g}
EKP10	601.93 ± 48.60^{c}	752.38 ± 85.86^{ef}
EKL0.1	385.51 ± 3.70^{b}	532.26 ± 42.34^{c}
EKL0.05	816.60 ± 2.04^{ed}	1127.96 ± 43.72^{g}
EKL0.001	617.10 ± 85.95^{f}	744.46 ± 78.65^{def}

Note: Data are presented in mean ± SD. Different superscript signs indicated a significant difference (p <0.05) in the Tukey HSD post hoc test. Note: EKP = Arabica coffee extract (ground coffee). EKL = coffee extract (liquid).

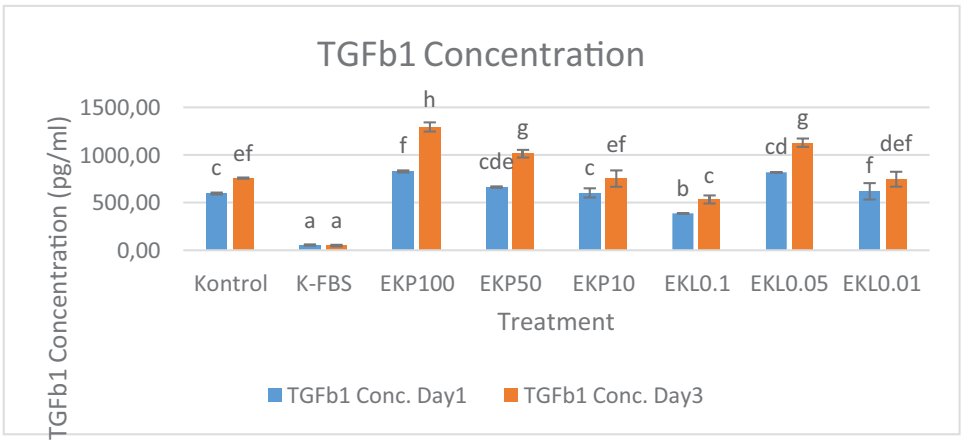

Figure 2: Effect of coffee extract on TGF-β1 concentration.

Note: EKP = Arabica coffee extract (ground coffee). EKL = coffee extract (liquid). The small letter follows the small letter in Table 2.

TABLE 3: bFGF concentration.

Sample	bFGF concentration	
	hour-24	hour-72
Control	484.89±35.48^{b}	835.78±25.53^{ef}
K-FBS	88.22±10.84^{a}	169.22±29.23^{a}
EKP100	697.11±20.00^{cd}	1158.00±34.60^{g}
EKP50	617.89±62.01^{c}	858.67±20.82^{f}
EKP10	504.89±24.30^{b}	745.00±67.88^{de}
EKL0.1	467.11±42.51^{b}	759.56±24.17^{def}
EKL0.05	682.78±29.36^{b}	856.2±42.46^{f}
EKL0.001	473.44±11.18^{cd}	702.56±45.27^{cd}

Note: Data are presented in mean ± SD. Different superscript signs indicated a significant difference (p <0.05) in the Tukey HSD post hoc test.

EKP = arabica coffee extract (ground coffee). EKL = coffee extract (liquid).

Note: The small letter follows the small letter in Table 3.

4.2. Neomycin-Bacitracin

Note: NB = Neomycin-Bacitracin.

Migrasi sel= Migration cell.

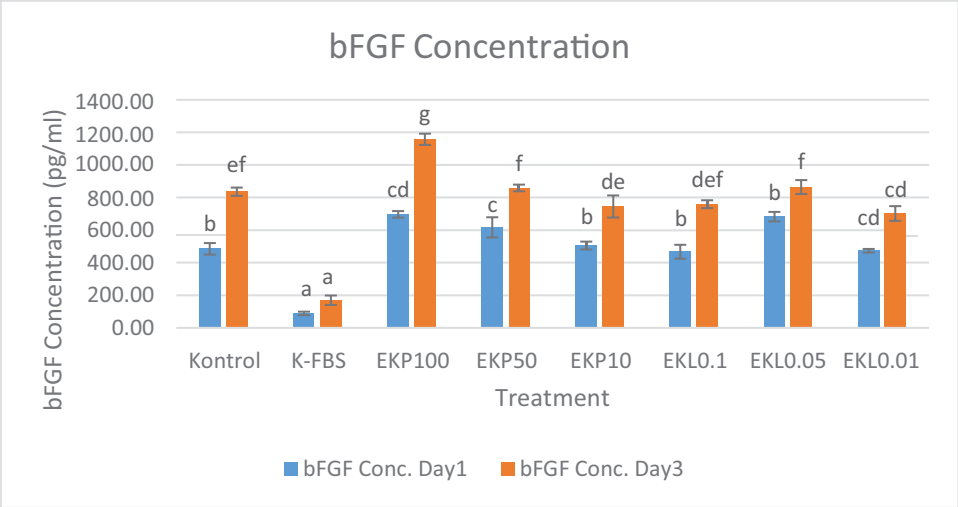

Figure 3: Effect of coffee extract on bFGF concentration. EKP = arabica coffee extract (ground coffee). EKL = coffee extract (liquid).

TABLE 4: Migration of Fibroblast cell culture.

		hour-0		hour-24
Sampel	**Area**	**Migration cell (%)**	**Area**	**Migration cell (%)**
Control	612028	0.00	292341	52.23
Control non-FBS	550337	0.00	454650	17.39
NB500	501061	0.00	377258	24.71
NB100	645406	0.00	461548	28.49
NB10	605989	0.00	439877	27.41

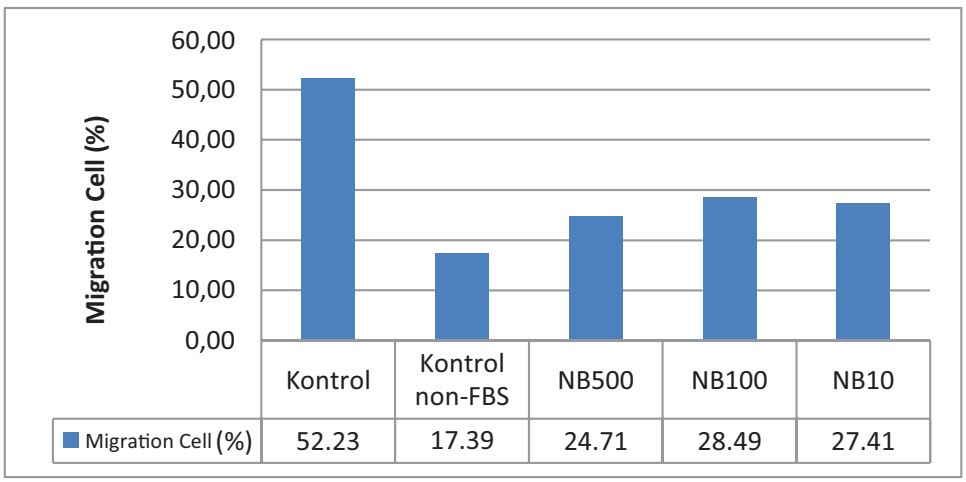

Figure 4: Cell migration by Neomycin-Bacitracin. Description: NB = Neomycin-Bacitracin.

Note: Data are presented in mean ± SD. Different superscript signs indicated a significant difference ($p < 0.05$) in the Tukey HSD post hoc test.

Note: a,b,c,d follow the Table 5.

TABLE 5: TGF-β concentration.

Sample	TGF-β1 concentration	
	Hour-24	**Hour-72**
Control	479.64±15.44^{b}	464.4±15.17^{b}
K non-FBS	54.95±7.40^{a}	50.45±9.64^{a}
NB500	445.25±17.19^{b}	474.12±2.83^{b}
NB100	523.43±2.23^{c}	560.19±19.27^{c}
NB10	525.28±5.30^{c}	655.72±30.38^{d}

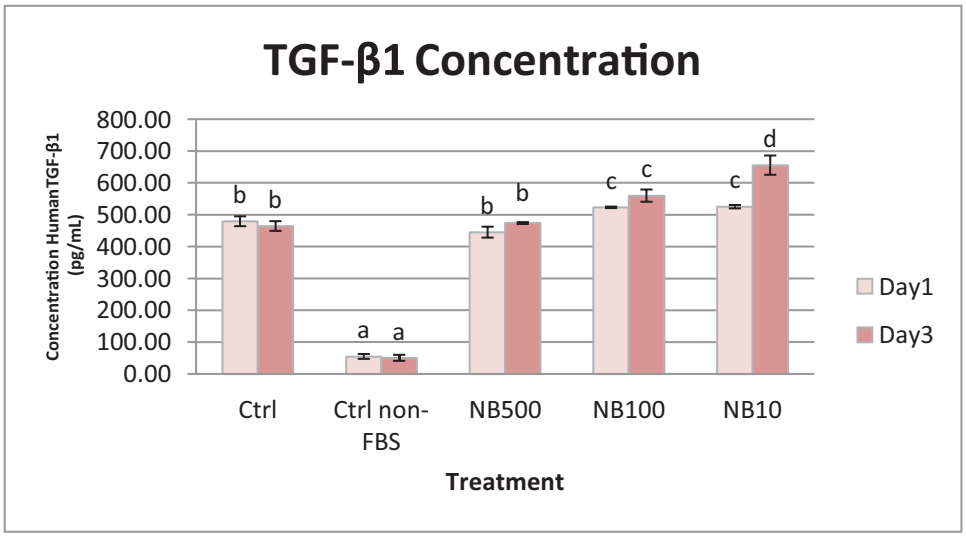

Figure 5: Effect of Neomycin-Bacitracin on TGF-β1 concentration.

TABLE 6: bFGF concentration.

Sample	bFGF concentration	
	hour-24	**hour-72**
Control	482.41±35.95^{b}	642.96±16.93^{bc}
K non-FBS	94.37±14.31^{a}	87.16±24.16^{a}
NB500	565.64±24.89^{b}	574.79±70.94^{b}
NB100	593.87±34.02^{b}	580.47±73.21^{b}
NB10	608.21±48b^{c}	778.26±135.77^{c}

Note: Data are presented in mean ± SD. Different superscript signs indicated a significant difference ($p < 0.05$) in the Tukey HSD post hoc test. NB = Neomycin-Bacitracin.

Note: a,b,c,d follow the Table 5.

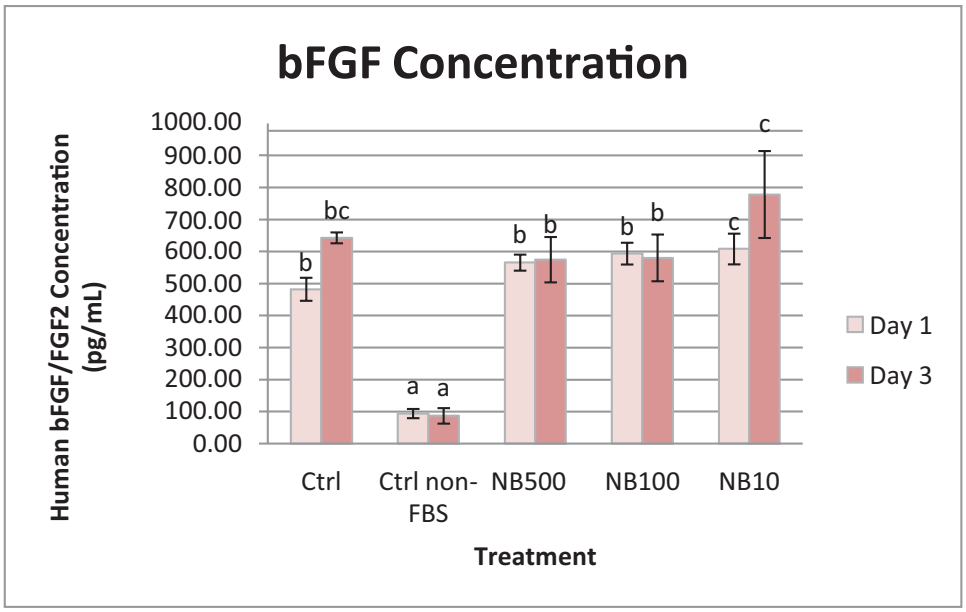

Figure 6: Effect of NB on bFGF concentration.

4.3. Coffee

Based on the test results, it is known that the coffee powder extract (EKP) at a concentration of 100 µg / mL has the best cell regeneration and anti-inflammatory potential in the formation of scar tissue. It is indicated by the percentage of BJ cell migration (63.51%), the highest concentration of TGF-β1 (1293.96 ± 48.44 pg / mL), and the highest concentration of bFGF (1158.00 ± 34.60 pg / mL).

The liquid coffee extract (EKL) at a concentration of 0.01% has the best potential to support cell regeneration as indicated by the percentage of BJ cell migration (52.79%). While the TGF-β1 concentration (1127.96 ± 43.72 pg / mL), and the highest concentration of bFGF / FGF2 (856.2 ± 42.46 pg / mL) were shown by giving liquid coffee extract at a concentration of 0.05%. But the value shown by EKP is better than EKL.

4.4. Neomycin-Bacitracin (NB)

The test results show that NB at a concentration of 100 µg / mL has the best potential to support cell regeneration in the formation of scar tissue. This is indicated by the percentage of BJ cell migration (28.49%). While the TGF-β1 concentration (655.72 ± 30.38) and the highest bFGF concentration (778.26 ± 135.77) were shown by the administration of NB10 µg / mL.

5. DISCUSSION

Coffee has become a popular drink globally, so coffee grounds are easy to find in Indonesia [1]. Apart from being used to aid wound healing, coffee is known as a safe skin softener because it improves skin microcirculation [5]. Coffee grounds for wound treatment have been described as a traditional method in many coffee producing countries. The method of wound treatment using coffee with satisfactory wound healing results is local wisdom in rural areas that continues to be used today. Research conducted since 2003 shows that coffee powder water extract has strong antibacterial, anti-inflammatory, and antioxidant properties, so that these reasons cause wounds to heal quickly [2]. Efficient and inexpensive to heal wounds, and the smell is an attraction that defeats other traditions such as honey, turmeric. Its use for various wounds, diabetes, acute, and chronic, causes fast, cheap, and painless healing, is not complicated and does not frighten wound sufferers. The use of coffee grounds for wounds is not scary, because the replacement is very long (can be 3-4 weeks), and especially there is no cleaning the wound 1-3 days or and many times. The innermost layer of the coffee powder is not removed, it will always protect the wound cells, so that the proliferation of cells is more secure and not damaged; thus, wound closure occurs quickly. The antibacterial properties of coffee make it impossible for bacteria to grow, so there will be no biofilm and resistant bacteria, thus preventing chronic wounds from occurring [1,2]. As a result of coffee research for wounds, the discovery of the basic principle to accelerate wound healing, namely that the cells on the surface of the wound tissue must be protected, it is prohibited to damage it by repeatedly cleaning the wound every 1-3 days. The best protection for these cells is coffee powder [2]. A thin layer of coffee powder is left on the wound surface, not replaced to protect the cells, thus ensuring fast healing and not causing trauma to the wound [3]. With only one wound cleansing (even if necessary) and protected by coffee grounds, healing is guaranteed, and scar tissue will grow well, and no lousy scarring, no hypertrophic or keloid scars. The antibiotic powder NB has long been known and is used to treat wounds. Its use of wounds should be changed every 1-2 days after cleaning (washing and removing old powder). Neomycin is made from the bacterium Streptomyces fradiae, so the manufacturing cost is relatively expensive. Also, Neomycin has a high prevalence of allergic contact dermatitis [5]. Bacitracin, derived from the bacterium Bacillus subtilis, is able to kill positive Gram microorganisms, especially Staphylococcus aureus and S. pyogenes, but generally it is unable to kill Gram negative bacteria. Systemic use is not recommended because it is toxic to the kidneys and veins, so it is only used for skin tissue surface infections.

The deterioration of bacitracin causes allergic contact dermatitis [5]. Fibroblasts are very much found in skin tissue. They have an essential function in the wound healing process, including their role in breaking down fibrin clots, forming extracellular matrix components, and collagen tissue structures, including a balance of tissue homeostasis to form cell growth. Cells fill the wound. Fibroblasts with keratinocytes, melanocytes, and epithelium / endothelium, are the cells responsible for restoring the main structure of skin tissue back to normal. Fibroblast cell culture was carried out by a scratch assay in the laboratory (in vitro) to measure cytokines that play a role in the healing process. This study will answer that coffee grounds heal faster wounds and do not cause excessive scarring [1,2]. Excessive scarring is found on hypertrophic scars or even keloids, due to repeated wound manipulation. Excessive scar tissue is indicated by the marker levels of TGF-β1 and bFGF [3-6]. Both markers have functioned in detection of anti-inflammatory and keep away of excessive scar [7,8]. In the process of wound healing, the inflammatory phase should be as short as possible. It will be difficult if the inflammatory phase is prolonged and even becomes obstructed due to the chronic inflammatory process. These events occur due to inflammation by bacteria that can reproduce, usually due to wound treatment that is unable to deal with the bacteria present. Chronic inflammatory events can occur due to low immunity or virulent bacteria. The advantage of using a wound dressing using coffee powder (or its extract) or NB powder, both of which have anti-inflammatory, even antibacterial, will shorten the inflammatory phase and accelerate wound healing [9]. Also, anti-inflammatory properties (coffee and NB) will suppress excessive fibrosis, which is a characteristic of scar tissue formation as a wound covering [9,10].

6. CONCLUSION

The results of cell migration, the concentration of TGF-β, bFGF also concluded that coffee extract and Neomycin-Bacitracin had anti-inflammatory properties. But coffee gives more robust results than Neomycin-Bacitracin in cell migration, anti-inflammatory. Showed that in the wound healing process Coffee and Neomycin-Bacitracin are useful in accelerating wound healing. Still, coffee is proven to have higher quality in wound healing than Neomycin-Bacitracin.

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References

[1] H.S. Yuwono, “The New Paradigm of Wound Management Using Coffee Powder.,” *Global Journal of Surgery*. vol. 2, no. 2, pp. 25–29, 2014

[2] Yuwono HS. *Kopi untuk mengobati luka*. Bandung: PT. Refika Aditama; 2013

[3] Gilbert RW, Vickaryous MK, Viloria-Petit AM. Signalling by Transforming Growth Factor Beta Isoforms in Wound Healing and Tissue Regeneration. J Dev Biol. 2016;4(2):1–21.

[4] Wang P, Shu B, Xu Y, Zhu J, Liu J, Zhou Z, et al. Basic fibroblast growth factor reduces scar by inhibiting the differentiation of epidermal stem cells to myofibroblasts via the Notch1/Jagged1 pathway. Stem Cell Res Ther. 2017 May;8(1):114.

[5] Shi HX, Lin C, Lin BB, Wang ZG, Zhang HY, Wu FZ, et al. The anti-scar effects of basic fibroblast growth factor on the wound repair in vitro and in vivo. PLoS One. 2013;8(4):e59966.

[6] Seo HY, Jung YA, Lee SH, Hwang JS, Park KG, Kim MK, et al. Kahweol decreases hepatic fibrosis by inhibiting the expression of connective tissue growth factor via the transforming growth factor-beta signaling pathway. Oncotarget. 2017 Aug;8(50):87086–94.

[7] Akita S, Akino K, Hirano A. Basic Fibroblast Growth Factor in Scarless Wound Healing. Adv Wound Care. 2013 Mar;2(2):44–9.

[8] Wang L, Ko CY, Meyers EE, Pedroja BS, Pelaez N, Bernstein AM. Concentration-dependent effects of transforming growth factor β1 on corneal wound healing. Mol Vis. 2011;17:2835–46.

[9] Yuwono HS. American Journal of Medical Case Reports. 2019;7(5):94–6.

[10] Garna H, Yuwono HS, Tejasari M. Vulnerable Wound-base Cells Protected by Coffee Powder to Better Healing. American Journal of Medical Case Reports. 2019;7(9):214–6.