

## Combination Effect of Argan Oil and Low Frequency Electromagnetic Field on Open Wound in Mice (Kesan Kombinasi Minyak Argan dan Gelombang Elektromagnetik Frekuensi Rendah terhadap Luka Terbuka pada Tikus)

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### ABSTRACT

*This study was conducted to evaluate the effect of argan oil with the exposure of low frequency electromagnetic field (EMF) on open wound healing in mice. Eighteen male mice (20-40 g) were divided into three groups: phosphate buffer saline (PBS) as negative control, solcoseryl gel as positive control, and argan oil with the exposure of low frequency EMF, 1.2 mT (treatment group). Full thickness wounds (4 mm diameter) were induced on the shaved dorsal of the mouse. All mice were sacrificed on day 12 after the final treatment. Macroscopic observation, wound contraction rate, histopathology analysis and total protein content were examined in this study. Results showed that wounds treated with argan oil and exposed to low frequency EMF has a significant increase in wound contraction rate ( $p < 0.05$ ) and total protein content ( $p < 0.05$ ). Moreover, histopathological analysis on the wound tissues displayed complete re-epithelization with thick and dense collagen fibers in the argan oil with low frequency EMF exposure treated group. In conclusion, topical treatment of argan oil with low frequency EMF exposure yield a better healing progress and showed the ability to accelerate wound healing.*

*Keywords: Argan oil; electromagnetic fields; open wound; wound healing; total protein*

### ABSTRAK

*Kajian ini dijalankan untuk menilai kesan minyak argan bersama dedahan medan elektromagnetik rendah dalam penyembuhan luka terbuka pada mencit. Sebanyak 18 ekor tikus jantan (20-40 g) telah dibahagikan kepada 3 kumpulan: penimbal fosfat (PBS) sebagai kawalan negatif, gel solcoseryl sebagai kawalan positif, dan minyak argan bersama dedahan EMF rendah, 1.2 mT sebagai kumpulan rawatan. Luka ketebalan penuh (4 mm diameter) telah diaruh ke atas bahagian dorsal kulit tikus. Kesemua tikus dikorbankan pada hari ke 12 setelah menerima rawatan terakhir. Pemerhatian makroskopik, kadar kontraksi luka, analisis histopatologi dan jumlah kandungan protein telah dianalisa untuk kajian ini. Hasil kajian ini menunjukkan peningkatan kadar kontraksi luka ( $p < 0.05$ ) dan jumlah kandungan protein ( $p < 0.05$ ) yang signifikan. Selain itu, analisis histopatologi tisu luka menunjukkan lapisan epitelium yang lengkap dengan serat kolagen yang tebal dan padat dalam kumpulan rawatan minyak argan bersama dedahan EMF rendah. Kesimpulannya, rawatan topikal minyak argan bersama dedahan EMF rendah menunjukkan perkembangan proses penyembuhan yang lebih baik dan berupaya untuk mempercepatkan penyembuhan luka.*

*Kata kunci: Minyak argan; medan elektromagnetik; luka terbuka; penyembuhan luka; jumlah protein*

### INTRODUCTION

Nowadays, the use of electrical devices is rising rapidly concomitant with the development of technology worldwide and also in line with human needs. Equipment such as microwave, laptop, radar, radio and cell phone is the source of the resulting electromagnetic fields (EMF), and this EMF is present around our environment without us being aware of it. There are many researches in the past that have shown the potential of low frequency EMF in medical and pharmacological functions such as cell differentiation, antioxidants and gene expression (Ross et al. 2015, Mahmoudinasab et al. 2016, Fang et al. 2016). Bertolino et al. (2006) revealed that exposure to magnetic fields can expedite the rate of wound healing, whilst study by Diniz et al. (2002) showed that the maturation of osteoblast is accelerated by EMF during the bone repair stage.

Argan oil, derives from the seed of the argan trees (*Argania spinosa*) contains high profile of fatty acids, sterol, squalene, tocopherols and phenolic compounds that have been shown to have various medicinal properties such as antioxidant and anticancer (Khallouki et al. 2003). Traditionally, argan oil has been used as an alternative treatment to several skin conditions including psoriasis, eczema and skin inflammation (Avsar et al. 2016).

There have been many studies that showing each effect of EMF and argan oil individually in the treatment of wound healing. However, the combination effects of argan oil and the exposure of EMF on open wound repairation steps has yet to be explored. Therefore, in this study we investigated the effects of topical argan oil under low frequency EMF exposure in the treatment of open wound on total protein expression, healing and contraction rates.

## METHODOLOGY

### PREPARATION OF ANIMALS AND OPEN WOUND MODEL

All animal handling procedures adhered to the regulation by the National University of Malaysia Animal Ethics Committee (UKMAEC) with approval number FSK/2016//YANTI/28-JAN./727-FEB.-2016-JAN.-2017. Eighteen (18) adult male ICR mice weighing 20-40 g were given standard mouse pellet diet and drinking water ad libitum. The mice were randomly divided into 3 groups: 1) Argan oil with the exposure of low frequency EMF, 1.2 mT (Kwan et al. 2015) treated group (n: 6 mice); 2) 10% solcoseryl gel as positive control group (n: 6 mice); and 3) phosphate buffered saline (PBS) negative control group (n: 6 mice). Pure (100%) argan oil extract in this study was collected from Technology Park Malaysia. Full-thickness 4 mm diameter wound was excised on the mouse's shaved dorsal by a sterile biopsy punch under general anesthesia (mixture of ketamine, zoletile and xylazine). Treatments for mice were carried out every 24 hours for 12 days. For the treated group, upon topical treatment with 1ml of argan oil (Avsar et al. 2016), the mice were placed in a solenoid and were exposed to low frequency EMF (1.2 mT) for 30 minutes. Animals were sacrificed with overdose of the general anesthesia on day 12 after macroscopic wound healing examination. The tissues from the excised sites were biopsied and part of the tissues was stored in 10% buffered formalin for histopathological analysis. Whilst another part of the tissues was kept at -80°C for total protein analysis.

### WOUND CONTRACTION MEASUREMENT

The diameter of wounded skin tissue was measured everyday by using a caliper measurement. The percentage of wound contraction was measured according to the following formula:

$$\text{Percentage of wound contraction (\%)} = \frac{(\text{initial wound size} - \text{specific day wound size})}{\text{Initial wound size}} \times 100$$

### HISTOPATHOLOGICAL ANALYSIS

Tissue samples containing part of the wound area from the skin were harvested on day 12 and transferred into 10% buffered formalin for histology processing. The tissues were embedded in paraffin wax and were sectioned to 5µm thickness before stained with hematoxylin and eosin (H&E). The tissue sections were observed under the light microscope.

### ANALYSIS OF TOTAL PROTEIN

Total protein expression in the wound was analysed according to the Bradford assay (1976). The wound skin tissue was homogenized in 0.1 M phosphate buffer (PBS), pH 7.4 at a ratio of 1:10 of the tissue weight to PBS. The

homogenate was centrifuged at 3000 rpm at 4°C for 5 minutes. The supernatant was then collected and further diluted with PBS for 1:100 ratio. The diluted supernatant (0.1 ml) was added into 5.0 ml Bradford reagent. Absorbance was measured at 595 nm by using spectrophotometer. The total protein content was calculated from a standard curve using 0, 0.2, 0.4, 0.6, 0.8, 1.0 mg/ml of bovine serum albumin (BSA).

### STATISTICAL ANALYSIS

IBM SPSS Statistics version 22 was used for statistical analysis. One-way ANOVA test was used to compare the percentage of wound contraction and total protein content between treated and control groups. Data was presented in mean ± SEM with the level of significance was set at  $p < 0.05$ .


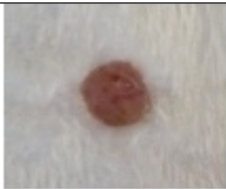

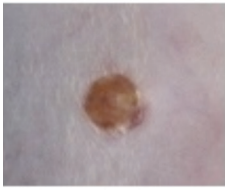
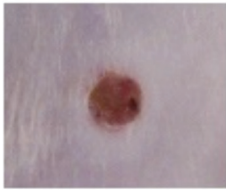
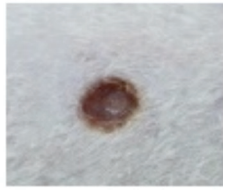


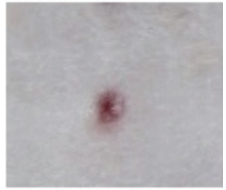





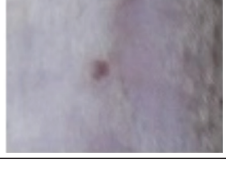
## RESULTS AND DISCUSSION

### RATE OF WOUND CONTRACTION

Macroscopic observation on the wound area in each group at different days was summarized in Table 1. There was no apparent changes in each group of mouse on the wound within 24 hours after full-thickness skin excision, except the wound area in the positive control and treated group which appeared to be slightly dry. At day-3, the formation of scab could be found only in the treated group. The scab could be formed as a result of the blood clots dried to protect the wound tissue underneath (Fitzpatrick et al. 2005). Study in the past has shown that low frequency expression of EMF on the wound could reduce the early inflammatory stage, thus the scab will be formed earlier leading to rapid healing process (Athanasίου et al. 2007). The wound size showed further contraction at day-6 and the scab appeared to slough off at day-9 for wound in the treated group. At day-12, the wound in treated group showed complete closure indicating the wound was considered as fully healed as compared to both control groups.

The wound contraction rate in each group was demonstrated in Figure 1. The wounds treated with argan oil with the exposure of EMF showed a significant increase in the percentage of wound contraction (\*, \*\*,  $p < 0.05$ ) on day-6 ( $60.0 \pm 5.6\%$ ), day-9 ( $93.7 \pm 2.6\%$ ) and day-12 ( $97.3 \pm 1.3\%$ ) as compared to both control groups. Wound contraction which is the key event in wound healing process is a reaction made by fibroblast cells that produce collagen and elastin fibers, and also organise the cellular matrix components within wound tissue (Carmen et al. 2011; Martin 1997). The cellular matrix components such as fibronectin and glycosaminoglycan would form a fibrin network and generate contractile force in wound (Martin 1997). Thus, results indicate that argan oil with low frequency EMF exposure could speed up the wound contraction event through recruitment of more fibroblasts cells to migrate to wound tissue area. Our data also showed

TABLE 1. Macroscopic observation on the wound area of experimental mice in each group shows wound contraction progression

Day	PBS Control group	10% <i>Solcoseryl</i> gel Control group	Argan oil with EMF treatment group
1			
3			
6			
9			
12			

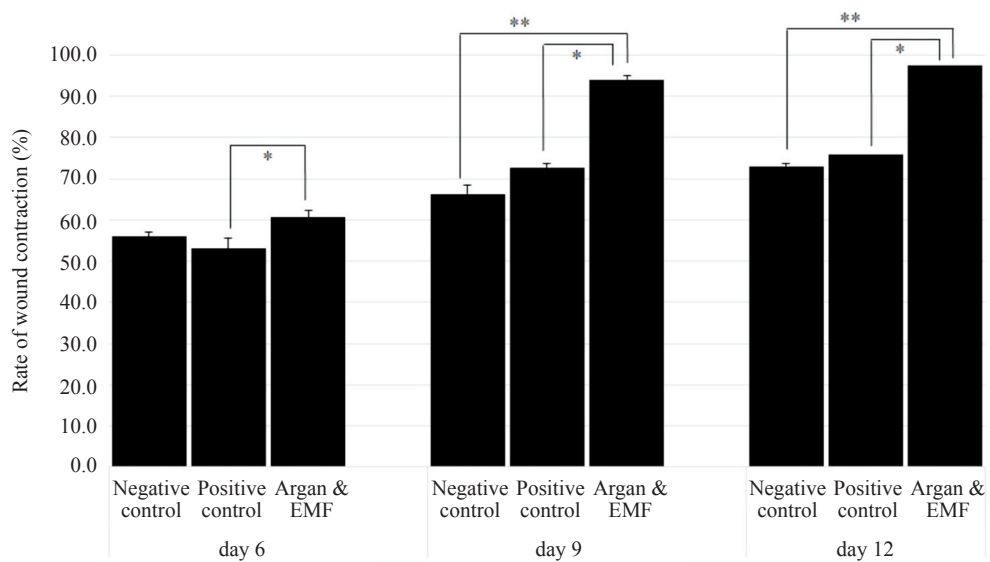


FIGURE 1. Effect of argan oil with low frequency EMF exposure on rate of wound contraction. Rate of wound contraction was measured as percentage (%) of reduction in the original wound diameter size (day-0). Treatment with argan oil and EMF exposure increased the rate of wound contraction (\*, \*\*,  $p < 0.05$ )

a significant wound contraction rate as early as day 6 with the combination of argan oil and low EMF exposure; In comparison to the past study by Avsar et al. (2016) using argan oil alone where the significant wound contraction rate (31%) occurred on day 7. Moreover, our findings of wound contraction rate support the macroscopic observations which indicate that wound healing process is faster in the treatment group rather than the control groups.

#### HISTOPATHOLOGICAL ANALYSIS

Figure 2 showed the histopathological changes of wounds harvested on day-12 and stained with H&E. Though all

groups demonstrated complete re-epithelization with keratinized layers of the wound area, wound healing process was still dominated in the treated group. According to our data, collagen fibers appeared more dense and well-arranged parallel to the epithelium in the treated group. Toste et al. (2002) showed in their study that wounds exposed to the EMF resulted in more deposition of collagen fibers and increase in collagen density. The combination of argan oil with EMF exposure in the wound area may generate an appropriate environment to accelerate the wound healing process.

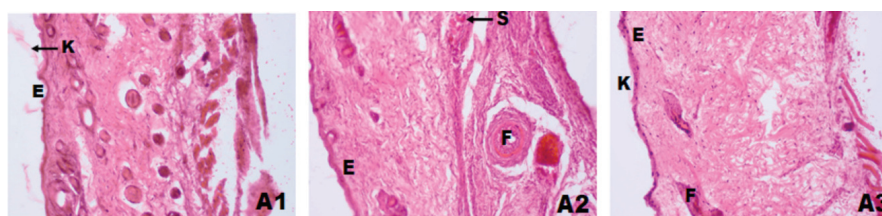


FIGURE 2. wound tissue section stained with H&E on the day-12. Each group displayed complete re-epithelization (A1: PBS negative control; A2: Solcoseryl gel positive control; A3: Argan oil with EMF). Treated group showed increased in collagen fibers deposition. (epithelium (E), keratin layer (K), blood cell (S), hair follicle (F))

#### TOTAL PROTEIN CONTENT

Total protein content of the wound tissues harvested from mice in each group on day-12 was measured using Bradford's assay. The total protein content is a parameter for the level of protein that signifies cellular proliferation of the wound (Teoh et al. 2009). According to our findings (Figure 3), treatment group with argan oil under exposure of EMF showed significant increase ( $p < 0.05$ ) of total protein content (0.53 mg/ml) as compared to the positive control

group (0.45 mg/ml). This may indicate that increased protein synthesis process and cellular proliferation occur in the treated group. Previous study showed that an extremely low frequency of EMF could activate more fibroblast cells migration to the wound area (Sunkari et al. 2011). As fibroblast cells act as the key player in the synthesis of collagen, thus, increase fibroblast cells migration could lead to high connective tissue proliferation and collagen matrix network (Ruszczak 2003).

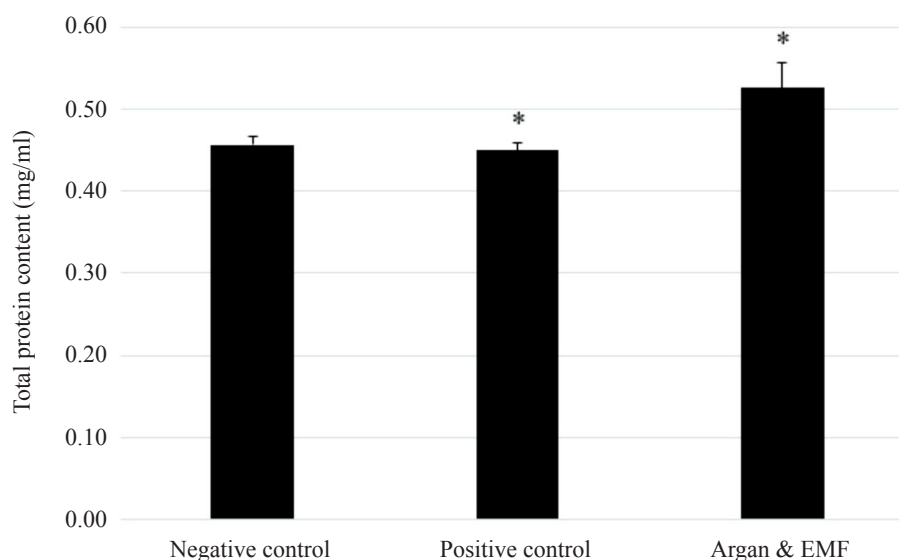


FIGURE 3. Total protein content in wound tissue harvested on day-12 from each group. There was significant increase in the total protein content in wound treated with argan oil and EMF exposure (\*,  $p < 0.05$ ). Data is expressed in mean  $\pm$  SEM with n: 6 per group

## CONCLUSION

In conclusion, application of topical argan oil with low frequency EMF (1.2 mT) exposure showed acceleration in the wound healing process and may have a potential influence in relation to wound contraction rate, fibroblast cells migration and total protein content. Moreover, our findings also have displayed that wounds treated with argan oil with low frequency EMF exposure yield a better healing progress as compared to the 10% solcoseryl gel. Further studies with molecular analysis such as on the fibroblast growth factor pathway may be needed to explore the mechanism of wound healing activity using argan oil under EMF exposure.

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