
# International Journal of Pharmacology

ISSN 1811-7775

science alert

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Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2024.527.535

Research Article

# Investigation of Some Histological, Physiological and Microbiological Properties of Honey Types and Vaseline as Medications of Second Degree Burns in Rats

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## Abstract

**Background and Objective:** Honey is a natural product with high osmolality and acidity, it affects the cytokines production by skin cells when applied as a coating treatment. The current study aimed to investigate some histological, physiological and microbiological properties of honey types and vaseline as medications for burns in rats. The irradiated honey types are named *Nigella sativa* honey (NSH), moringa honey (MOH), sidr honey (SIH) and pumpkin honey (PUH), respectively. **Materials and Methods:** The 60 adult male albino rats were divided equally into 6 sets. Second-degree burns were induced, while animals in (3, 4, 5 and 6) groups were treated with irradiated honey (NSH, MOH, SIH and PUH) as ointment coatings daily, while group 1 was treated with vaseline, respectively. Clinical assessments included histopathological analysis such as the fibroblasts and vessel density ratios. The wound diameter and color of the dermis were evaluated. The microbial infection was detected against *Escherichia coli* on burned wounds. Each wound was evaluated on days 0, 5, 10 and 15 until the full healing. **Results:** The fibroblast ratio on the 15th day was recorded at 68.44, 72.99, 74.53 and 63.70 for (NSH, MOH, SIH and PUH) compared to the vaseline (77.21). The highest vessel density ratio was detected for the treated rats with MOH (15.87). The wound diameter reached the maximum diameter contraction on treated rats with PUH (2.37 mm). The microbial population against *Escherichia coli* reached the maximum on treated rats with vaseline group (8.89 Log CFU/mL). **Conclusion:** The topical use of PUH followed by SIH and NSH enhanced the healing processes of burned rats.

Key words: Burning skin, honey, natural products, healing wounds, microbial loud

Citation: Kadi, R.H., R. Sami, M. Almatrafi, S. Harara and R.A. Al-Eisa *et al*., 2024. Investigation of some histological, physiological and microbiological properties of honey types and vaseline as medications of second degree burns in rats. Int. J. Pharmacol., 20: 527-535.

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Funding: The research was funded by Taif University, Saudi Arabia, Project No. (TU-DSPP-2024-10).

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Burns are defined as injuries to the skin caused by heat, radiation, radioactivity, electricity, friction or even chemicals[1]. Burns are a worldwide problem and can be affected according to several issues such as the degree, etiology and the organs convoluted[2]. Healing wounds is one of the existing problems, while research is going on in those areas. The healing burns can pass throw different phases starting with tissue injury with inflammations, homeostasis and proliferations, while reaching reactive oxygen species is very important to be controlled[3]. The restoration of that barrier is very important to avoid additional damage or infections. For some superficial burns, various medications are available at high prices and may cause adverse effects, especially in some poor communities[4]. Therefore, a serious need for wound dressings and topical products to boost healing and decrease scarring. Using some available, safe, effective and natural products such as honey is very important to reach the remodeling phases and repair wounds[5]. Honey as a viscous food substance can be varied according to several factors such as climatic diversity and plant origins which can be transformed by bees[6]. Some research works focused on the nutritional values, acidity, autolytic debridement activation, the increase of healing processing with the efficient activities of expansion of blood vessels, antioxidant, anti-allergic, antimicrobial, antithrombotic and anti-inflammatory[7,8]. It can enhance the production of proinflammatory cytokines secretion and cell proliferation[9,10]. Several microbial infections such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* can be caused by open injuries[11]. Subrahmanyam[12], studied the bacteriostatic and bactericidal properties of honey due to its osmolarity. The contemned wounds may lead to prolonging the period of healing processes and the formation of scars and other complications[13]. Several types of honey are well-known for medical uses such as Manuka and *Acacia gerrardii* Benth due to their therapeutic properties.

However, the therapeutic potential in burn healing has not been investigated yet for other honey types. The current research aimed to investigate some histological, physiological and microbiological properties of honey types and vaseline as medications for burns in rats.

## MATERIALS AND METHODS

**Study area:** The comparative experimental study was carried out from September to December, 2023 in the Department of Food Science and Nutrition, College of Sciences, Taif University, Saudi Arabia. After obtaining the approval from the Institutional Ethics Committee (HAO-02-T-105).

**Experimental rats:** The rats were kept in clean metabolic cages at 25°C with a humidity of 70% and a light and dark cycle of 12 to 12 hrs. They were housed for 7 days to adapt before research work. They were provided with a basic diet and drinking water *ad libitum* for 15 days for the medication.

**Study design and irradiation processes of honey types:** The 60 adult male albino rats weighing 300-330 g were sourced from the Animal House Center in Jeddah. Randomly selected animals were divided equally into 6 sets, each set (n = 10). Animals in group 1 were considered as vaseline treatment, group 2 was considered as the negative control (-) and groups 3, 4, 5 and 6 were considered as experimental honey groups designated by their origin and named as *Nigella sativa* honey (NSH), moringa honey (MOH), sidr honey (SIH) and pumpkin honey (PUH), respectively. Four types of honey were obtained from Elshifa in Taif City, Saudi Arabia for treating rats. Approximately 2 kg from each type were subjected to a cobalt-60 irradiator with a Co-60 source measuring 24,000 at King Abdulaziz City for Science and Technology, Saudi Arabia.

**Experimental burn processes:** Wounds of 400 $mm^2$ were created by marking, shaving with depilatory cream and disinfecting with the methylene blue on the animal's dorsum under 40 mg/kg anaesthesia injection of sodium dipyrone then the addition of 200 mg/kg in the drinking water[14]. Second-degree burns were induced with hot aluminum plaques for 10 sec. Animals in (3, 4, 5 and 6) groups were treated with (NSH, MOH, SIH and PUH) as ointment coatings daily, while group 1 was treated with vaseline, respectively. The negative control (-) was left without any treatment.

**Clinical assessments:** The clinical assessments included histopathological analysis such as the fibroblasts and vessel density ratios. The wound diameter and color of the dermis were evaluated. The microbial infection was detected against *E. coli* on burned wounds. Each wound was evaluated on days 0, 5, 10 and 15 until the full healing. Figure 1 shows the summary of the comparative experimental study.

**Evaluation of the fibroblasts and vessel density ratios:** For the histopathological analysis of rat groups, samples were taken under general anesthesia by punch devices that

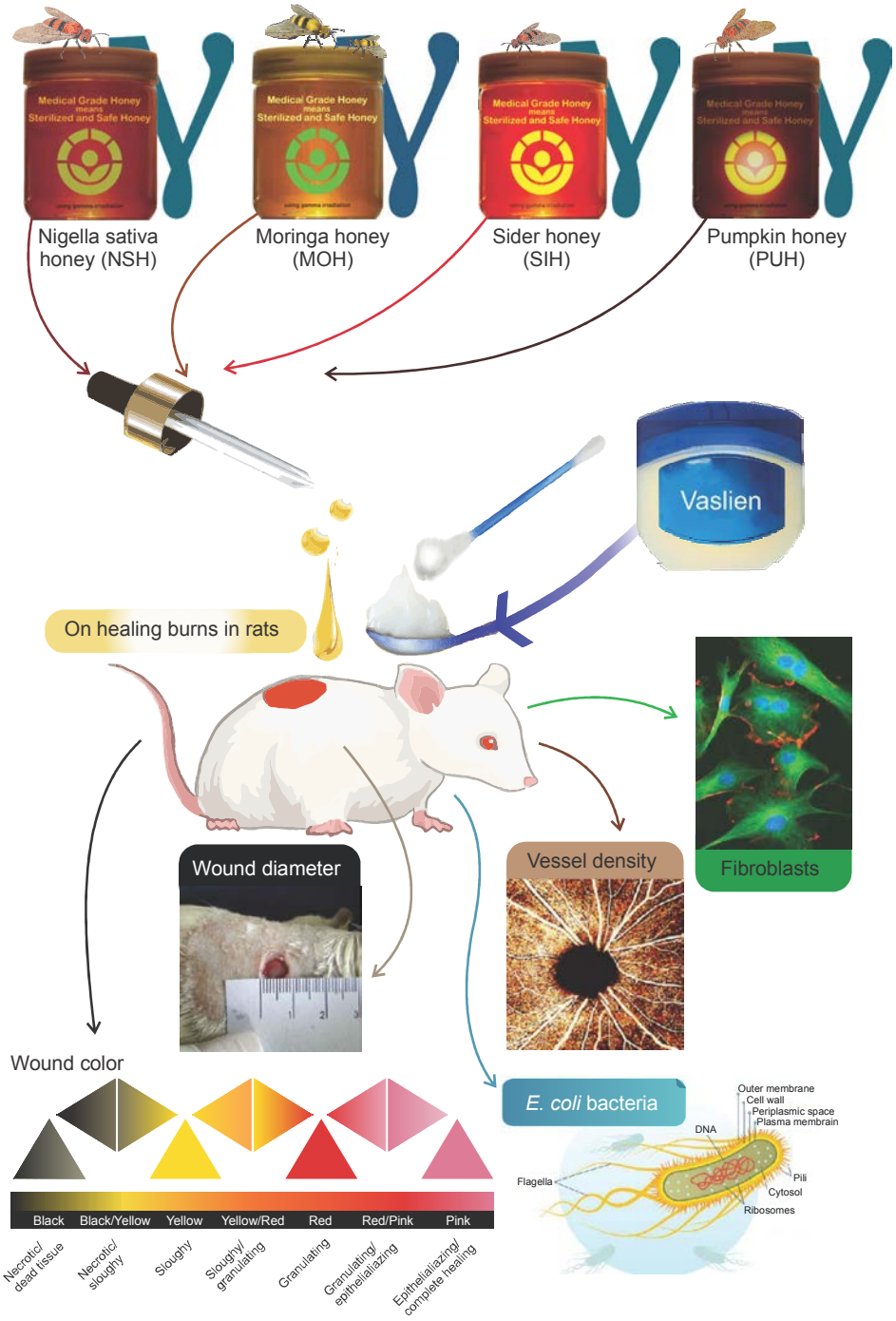


Fig. 1: Summary of the comparative experimental study

contained parts of the burns with their surrounding skins. The stained sections were detected on the 15th day for the fibroblasts and vessel density ratios by a Leica DM500 magnification light microscope under 400× magnification resolution then the images captured by A-352 Moticam camera were processed by LAS CORE™ software[15].

**Evaluation of the wound diameter and color:** The wound diameter was expressed in (mm) by using a digital caliper on days 0, 5, 10 and 15 until the healing was complete[16]. The wound color was visually evaluated as the following scale according to Keast *et al.*[17], who reported a (1) Dark gray color, (2) Creamy color, (3) Reddish color and (4) Bright red color.

**Evaluation of the microbiological infection:** The burned areas were collected and several dilutions were prepared to reach 10-fold and immediately cultured on Mueller Hinton agar plates. The microbial infection against *E. coli* and incubated at 37°C for 24 hrs. The results were expressed in (Log CFU/mL)[18].

**Statistical analysis:** Statistical evaluations of the quantitative results were applied to SPSS (version 11.5, Chicago, Illinois) and ANOVA followed by Duncan's t-test. Statistical significance was considered significant at $p<0.05$.

## RESULTS AND DISCUSSION

**Effect of various coatings on fibroblasts ratio:** Higher fibroblast ratio features to complete healing were observed. According to the results in Fig. 2, the fibroblasts ratio on the 15th day recorded 68.44, 72.99, 74.53 and 63.70 for (NSH, MOH, SIH and PUH) compared to the vaseline group which reached 77.21. Figure 3 presents the microscopic evaluations of the burned areas. The current results were in agreement with several published reports showing the healing mechanism of honey on burned areas by the differentiation and proliferation of the monocytes and fibroblasts[19,20]. Efem[21] reported the effect of honey on wound closure can be due to the bioactive ingredients. Also, the presence of hydrogen peroxide may enhance the proliferation of the fibroblasts. Honey can increase the formation of macrophages, fibroblast growth and the endothelial cells that make up the extracellular matrix and neovascular[22]. Thus, using honey as a coating treatment especially SIH and PUH types was shown to be effective as the reinforcement of the abdominal wall in case of burning skin.

**Effect of various coatings on vessel density ratio:** Based on the results in Fig. 4, using honey types and vaseline as coatings for determining the vessel density ratio on the 15th day, it was noticed that honey enhanced the healing process of burns. The highest vessel density ratio was detected for the treated rats with MOH (15.87), followed by PUH (15.77), SIH (13.11) and NSH (11.37), while the vaseline rat group (5.44) was the lowest ratio. Flavonoid and Hydrogen Peroxide ($H_2O_2$) components in honey may increase the production of angiogenesis and keratinocyte[23]. Also, $H_2O_2$ with the help of essential components such as saponins, genistein and naringin may attract the leukocytes, decrease pain and enhance the healing processes[24,25].

**Effect of various coatings on wound diameter:** All measurements of wound diameter were recorded on days 0, 5, 10 and 15 until the healing was complete. The burned areas treated with various coatings on all rat groups presented a continuous burn edge contraction during the full treatment, Fig. 5. While, on the 15th day, the wound diameter reached the maximum diameter contraction on treated rats with PUH (2.37 mm), followed by NSH (3.95 mm), SIH (4.98 mm) and MOH (5.63 mm), while vaseline rat group reached (7.49 mm). The results for wound diameter in the current study were in agreement with Dunford *et al.*[26], who reported the important role of honey in increasing wound contraction

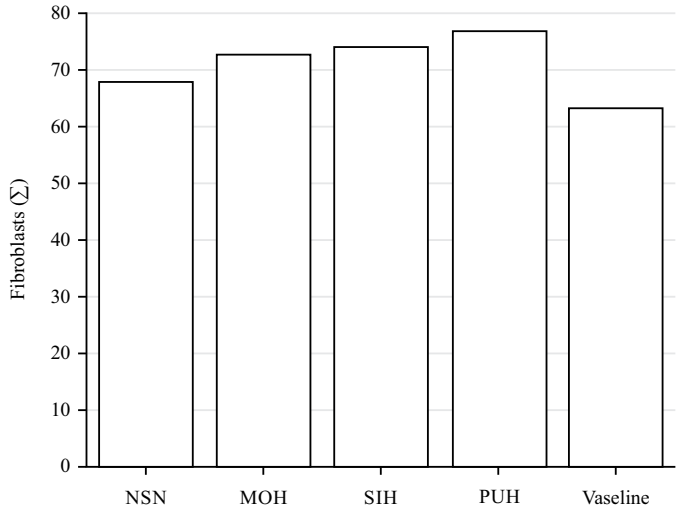


Fig. 2: Effect of various coatings on fibroblasts ratio based on their spindle shape combined with positive staining for the mesenchymal marker vimentin and the absence of staining for epithelial

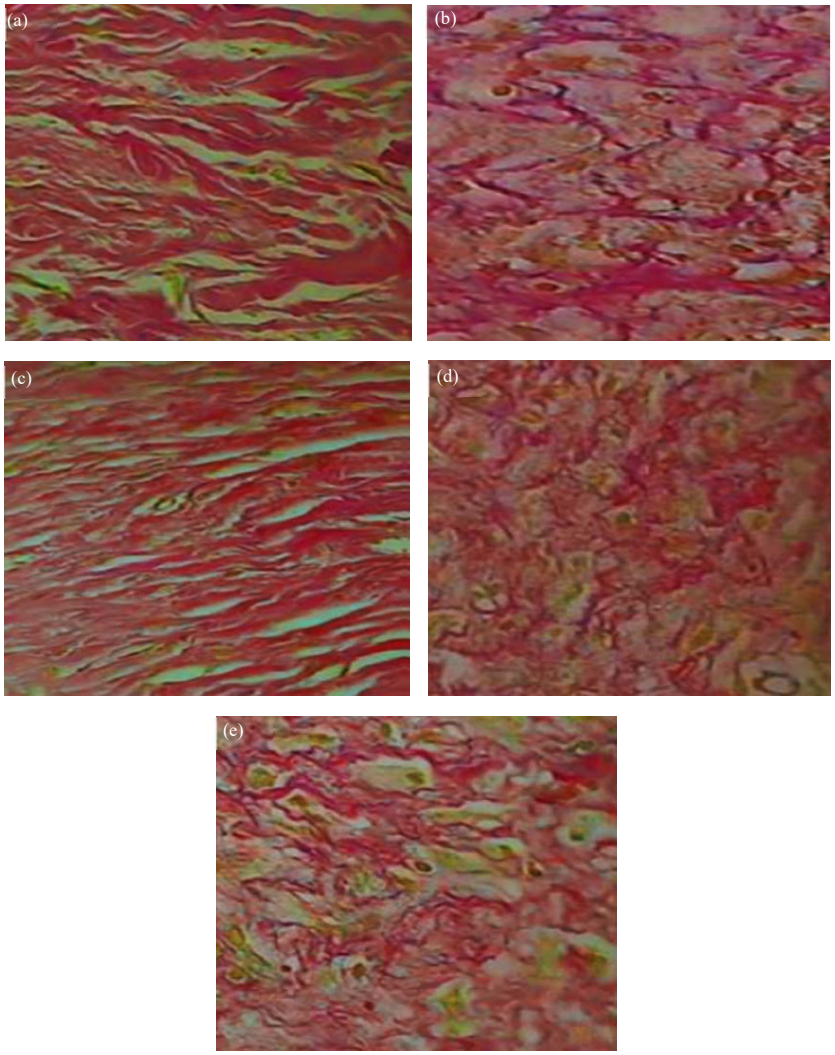


Fig. 3(a-e): Microscopic evaluations of the burned areas, (a) PUH, (b) NSH, (c) SIH, (d) MOH and (e) Vaseline

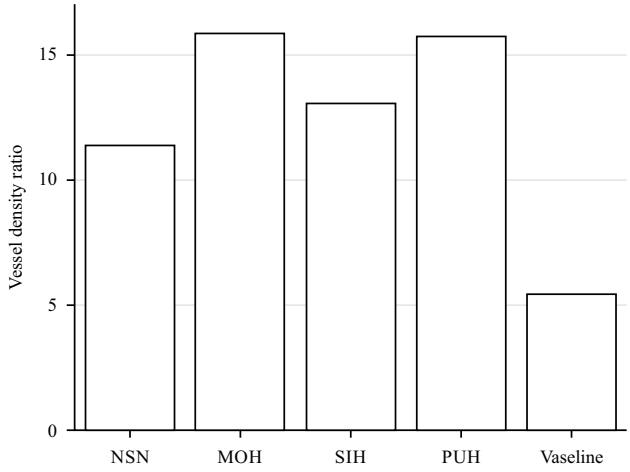


Fig. 4: Effect of various coatings on vessel density ratio

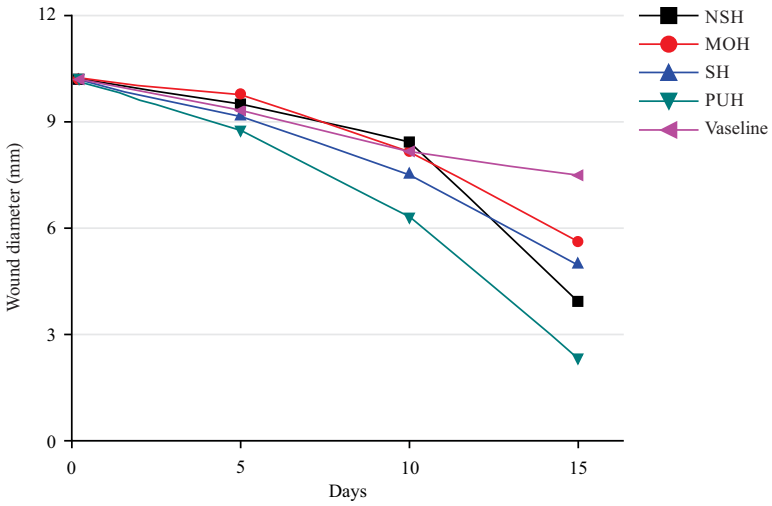


Fig. 5: Effect of various coatings on wound diameter

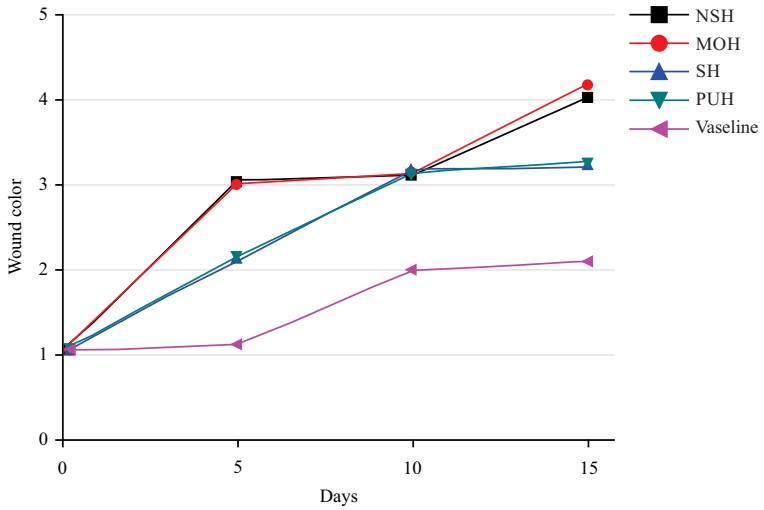


Fig. 6: Effect of various coatings on wound color

and granulation tissues. Various honey types can be varied in healing wounds, Yaghoobi *et al.*[27], reported the efficiency of honey as an anti-bacterial, anti-inflammatory anti-oxidant and anti-viral agent. Molan[28], reported that Manuka honey can cure chronic ulcers, burns and methicillin-resistant *Staphylococcus aureus* infections with antimicrobial activity against *Pseudomonas aeruginosa*, Gram-negative and positive organisms. Al-Eisa *et al.*[29], reported that honey can increase wound contraction due to several factors such as catalase, hypertonicity and low acidity.

**Effect of various coatings on wound color:** The wound color was detected on days 0, 5, 10 and 15 until the healing was complete in Fig. 6. The vaseline group achieved a dark creamy color (2.11), SIH and PUH achieved more reddish color (3.23 and 3.28), while MOH and NSH achieved bright red color (4.18 and 4.04), after 15 days of treatment, respectively. It was noticed that the wound diameter was reduced and became bright red, this was in agreement with the study of Emsen[30]. Therefore, using honey as a coating treatment especially MOH and NSH types was effective in reducing the anti-inflammatory activities, stimulating growth and repairing tissues.

**Effect of various coatings on *Escherichia coli* population:** Skin swabs on the burned areas were often detected to evaluate any microbial population. It was noticed that no obvious microbial growth during 15 days, which means no infection occurred in the burned areas, especially honey groups. The results of the microbial population against *E. coli* were presented in Fig. 7. On the 15th day, the microbial population against *E. coli* reached the maximum on treated rats with vaseline group (8.89 Log CFU/mL), followed by

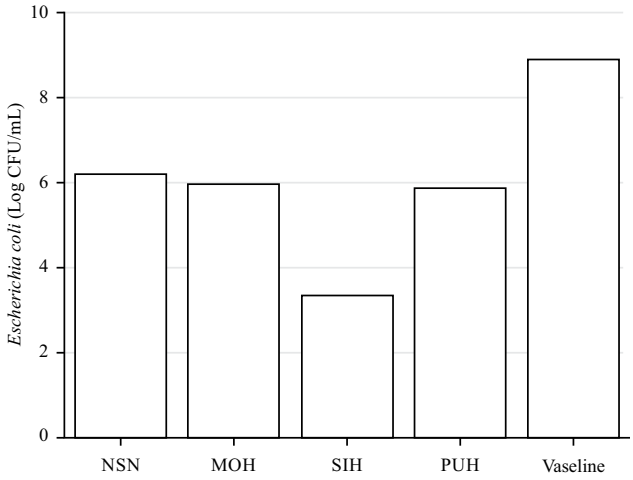


Fig. 7: Effect of various coatings on *Escherichia coli* population

NSH (6.17 Log CFU/mL), MOH (5.95 Log CFU/mL) and PUH (5.87 Log CFU/mL), while SIH rat group reached the minimum population (3.33 Log CFU/mL). Baakdah *et al.*[31], reported the efficacy of the antibacterial activities by using honey as a coating treatment to sterilize wounds and stimulate the formation of healthy tissues. While, the low acidity of honey cannot provide a good condition for microbial growth[32]. Al-Eisa *et al.*[33], reported the same conclusion of using honey to reduce inflammation, TNF-α immunology responses and microbial loud. Al-Waili[34], reported the efficiency of 2 types of honey Manuka and Tualang honey against the presence of *Pseudomonas aeruginosa* in injuries. Sharma[35], applied honey as a coating treatment against the presence of *Klebsiella pneumonia* on human patients. *Klebsiella pneumonia* is considered one of the most common organisms, which has a resistance to several antibiotics.

In the current research work study, we evaluated the beneficial possible of 4 honey types and vaseline as coatings for burned areas. The results established the useful effects of those honey types, each type was useful in a variety of some aspects in healing wounds. Overall, the outcome suggest that honey may supply as an important ointment for burns and inhibiting microbial loud.

## CONCLUSION

The current results indicated that pumpkin honey can be recommended as an alternative to healing wounds by increasing the ratios of fibroblasts and vessel density with a reduction of the wound diameter. However, *Nigella sativa* honey exhibits positive effects of the microbial population against *Escherichia coli*. Sidr honey reported the best appearance in the wound color. Additional investigations and mainly anthropological studies are needed to evaluate those components in pumpkin honey and other honey types.

## SIGNIFICANCE STATEMENT

Healing wounds is one of the existing problems, therefore, a serious need for wound dressings and some topical products to boost up healings. Using some obtainable, safe, efficient and natural products such as honey types is incredibly significant for reaching the remodeling phases and repairing wounds. The results of the current research work provided some pharmacological properties of using honey and vaseline as coatings for burns. However, the results recommended extra studies *in vivo*, the *in vitro* data are needed for healing burns.

## ACKNOWLEDGMENT

The authors extend their appreciation to Taif University, Saudi Arabia, for supporting this work through project number (TU-DSPP-2024-10).

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