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Burn Wound Healing Effect and Hair Growth Promoting Activity of *Lawsonia inermis* L. and Honey in *Oryctolagus cuniculus* Rabbits

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Abstract: This study aimed to assess *Lawsonia inermis* L. and honey mixture effects on burn wounds and hair growth in rabbits. Nine male *Oryctolagus cuniculus* local rabbits were allocated randomly in 3 groups. After antisepsis and local anesthesia, four circular burns were created on the animal's backs. Immediately after burning, the wounds were covered with honey in three rabbits (HON group), another group was treated first with honey and then *L. inermis* powder was added on honey (LI_HON group). Three untreated animals were used as control (CRL group). The treatment of rabbits was applied once daily (6/7 days) until 24th day post burns. The animals were observed for their general state, aspects of wounds and the healing times were also recorded. At 35th day post burns, the hair growth was investigated in the different groups. LI_HON group has recorded the best results compared to other groups; a short significant necrosis duration (LI_HON Vs CRL, $p<0.001$; LI_Hon Vs HON, $p<0.05$), precocious start of 1st crust detachment (LI_HON Vs CRL, $p<0.05$; LI_Hon Vs HON, $p<0.001$), a quick complete detachment of the first crust (LI_HON Vs CRL, $p>0.05$; LI_Hon Vs HON, $p<0.05$) and a significant reduction of healing time (LI_HON Vs CRL, $p<0.05$; LI_Hon Vs HON, $p<0.01$). LI-HON group has shown the best promoting activity of hair growth in term of recovered surface percentage (LI_HON Vs CRL, $p = 9{,}13038E^{-7}$; LI_Hon Vs HON, $P = 1{,}70745E^{-7}$) and hair length (LI_HON Vs CRL, $p<0.01$; LI_Hon Vs HON, $p\leq0.001$). The study concludes that the topic use of *L. inermis* powder and honey simultaneously accelerates burn wound healing process in rabbit's model. Henna has also shown a remarkable hair growth promoting activity in term of hair recovered area and hair length.

Keywords: *Lawsonia inermis* L., Honey, Burns, Wound Healing, Hair Growth

Introduction

Wound healing is a natural and dynamic process; it occurs in three fundamental steps including: inflammation, proliferative and remodeling phases (Wulff and Wilgus, 2013). Inflammation includes coagulation and inflammatory cell recruitment. The fibrin clot formed contains fibrin molecules, fibronectin, vitronectin and thrombospondins; it hosts the active arriving cells and different cytokines and growth factors (Reviewed by Broughton *et al.*, 2006). Macrophages are present throughout all stages of the healing process, producing a variety of substances that regulate healing including growth factors, prostaglandins and complement factors (Nathan, 1987). Numerous growth factors, cytokines and chemokines implicated in the execution and regulation of the wound healing stages were investigated. (Barrientos *et al.*, 2008). The physiological processes of angiogenesis, granulation tissue formation, epithelialization, collagen synthesis and wound

contraction characterized the proliferative phase (Broughton *et al.*, 2006). The wound healing cascade finishes by remodeling phase which occurs in three weeks to several years according to wound's complexity (Clark, 1988). The apoptosis of cells in this step prevent the appearance of hypertrophic scar or keloïd; in normal conditions a mature wound was formed and characterized as avascular and acellular (Broughton *et al.*, 2006).

According to Khorasani *et al.* (2008), thermal burn injury represents a major cause of death and disability and may cause a high costs in health care. The conventional drugs used in the management of burn injuries were reported to exert unwanted side effects (Fan *et al.*, 2015).

Many of today's modern drugs have their origin in traditional plant medicine (Blanks *et al.*, 1998). The use of plants for the healing purposes cited around 3000 B. C, in China, Egypt and subcontinent (Morse, 1934).

Henna is a tall shrub or small flowering tree. The scientific name of this plant is *Lawsonia inermis* L., which belongs to Lythraceae family. It is a shrub that grows in North Africa and Middle East (Gibbons *et al.*, 2005). Henna is native to a number of tropical regions in Asia, Northern Africa and Australia. It is naturalized and cultivated in the tropics of America, Egypt, India and parts of the Middle East (Muhammad and Muhammad, 2005). The different parts of henna have been reported to exert antioxidant, hypoglycemic, antidiabetic, hepatoprotective, antibacterial, antifungal, anticancer and wound healing properties (Majtan *et al.*, 2013; Hadagali and Chua, 2014; Rajashri and Sachin, 2014; El Bergadi *et al.*, 2015; Devasvaran and Yong, 2016; Mohamed *et al.*, 2016).

Honey is a natural product of *Apis mellifera*, it is prescribed in external applications and in the management of wound healing since the ancient times, from ancient Egypt, Chinese, Indian, to Greek civilizations) (Siedentopp, 2009). Several studies have focused the pharmacological properties of honey including antibacterial activities (Molan, 1992; Khoo *et al.*, 2010; Al-Nahari *et al.*, 2015), antifungal (Irish *et al.*, 2006; Boukraa *et al.*, 2008), anti-Inflammatory (Beretta *et al*., 2010), antioxidant (Al-Mamary *et al.*, 2002; Gheldof and Engeseth, 2002), hypoglycemic and hypolipidemic (Al-Waili, 2004), healing activities (Khoo *et al.*, 2010), cardioactive, vasoactive effects (Rakha *et al.*, 2008) and anticancer activity (Fernandez-Cabezudo *et al.*, 2013; Erejuwa *et al.*, 2014).

The aim of the present study is to evaluate the effect of a combination of honey bee and *Lawsonia inermis* on burn wound healing and to investigate the possible hair growth promoting activity in rabbit's model.

Materials and Methods

Drugs

200 g of commercial *Lawsonia inermis* L. powder was purchased from local market. It is an imported product from Pakistan under the name "Special mehndi", fabrication date: October, 2013 and expiration date: September, 2018. Honey was obtained from a local bee keeper from Collo, East of Algeria.

Animals and Housing Conditions

The study was conducted on 9 healthy male, adult, *Oryctolagus cuniculus* local rabbits weighing 1500 to 1800 g at the beginning of experiment. The animals were kept in normal conditions of temperature and lighting (12 h light: 12 h dark) and given laboratory food and water, *ad libitum* during the study. The experimental protocol was approved by the scientific committee of the department of sciences of nature and life, faculty of sciences, University of august, 20th 1955, skikda, Algeria.

Fig. 1. Localisation of cranial and caudal burns

Experimental Design

Three days before burning, the animal backs were clipped and a depilatory cream was used to remove all the hairs from the rabbit's skin. On day zero, a local anesthesia was practiced for all rabbits using xylocaïn 2% (s/c) after disinfectant by surgical alcohol 70%. Then, four burns of identical size (22 mm in diameter) were created on the back of each animal, two cranially (left and right) and two caudally (left and right) (Fig. 1), by a cylinder metal weighing 200 g immersed in prior in boiled water for 3 min and maintained on animal skin 15 sec (Djerrou *et al.*, 2010).

Treatment and Assessment of Healing Process

The animals were randomly allocated into three groups, the first untreated was used as control (CRL group), in the second 3 rabbits (HON group), the burns were immediately covered by a thin layer of honey (≈1 mm), the third group was treated as HON group and then honey was covered by 0.5 g of *Lawsonia inermis* L. powder. All these drugs were applied topically slowly and they were repeated once daily until 24th day post burns for non healed burns. The clinical aspects of wounds and the different healing times were noted. Photographs were taken from the wounds on days 0, 8, 12 and 20.

Hair Growth Assessment

At 35 day post burns, the hair growth was compared in the different rabbits; the percentages of recovered areas were calculated and the lengths of back's new hair were compared with the lengths of hairs in the left and right normal sides of animals.

Statistical Analysis

The results were expressed as mean with their variance. One-way Analysis Of Variance (ANOVA) was used to compare the different group means. A value of $p<0.05$ was considered statistically significant.

Results

Generally, there was a favorable trend towards healing in different rabbits either treated or untreated. Body weight of the animals has recorded a significant decrease during the first 15 days after burns in different groups; noting a return to the original state that was obtained within 2 weeks. One rabbit from LI-HON group showed a dermal reaction following application of the depilatory and that has healed at the 6th day post burns (Fig. 2), the same rabbit also expressed a skin thickening 15 days after the beginning of the application of honey and henna mixture.

Healing Process

The results of healing process recorded in Table 1 and Fig. 3 showed that application of *Lawsonia inermis* and honey simultaneously stimulated significantly the healing process in its different stages compared to honey applied only or untreated animals. HON group has enregistred a significant reduction ($p<0.005$) of necrosis duration compared to CRL group but no sgnificant differences were observed between these two groups in the processes of crust detachment or the total healing time. LI_HON group has recorded the best results compared to other groups; a short significant necrosis duration (LI_HON Vs CRL, $p<0.001$; LI_Hon Vs HON, $p<0.05$), precocious start of 1st crust detachment (LI_HON Vs CRL, $p<0.05$; LI_Hon Vs HON, $p<0.001$), a quick complete detachment of the first crust (LI_HON Vs CRL, $p>0.05$; LI_Hon Vs HON, $p<0.05$) and a significant reduction of healing time (LI_HON Vs CRL, $p<0.05$; LI_Hon vs HON, $p<0.01$).

Hair Growth

The results relative to hair growth registred in Table 2 and illustrated by Fig. 4 showed a real promoting activity of hair growth in LI-HON group in term of recovered surface percentage (LI_HON Vs CRL, $p = 9{,}13038E^{-7}$; LI_Hon Vs HON, $P = 1{,}70745E^{-7}$), the untreated rabbits have recorded a significant hair growth compared to HON group ($p<0.01$), however, there was no significant difference between these two groups in term of new hair length ($p>0.05$). In LI_HON group, the new hair length has reached a very significant difference compared to other groups (LI_HON Vs CRL, $p<0.01$; LI_Hon Vs HON, $p\leq0.001$).

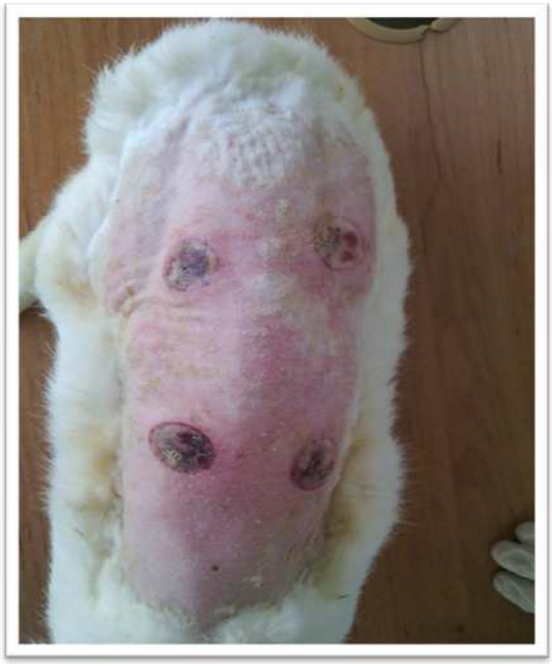

Fig. 2. Slight erythema on the back of a rabbit from *Lawsonia inermis*_honey group at the 4th day post burn

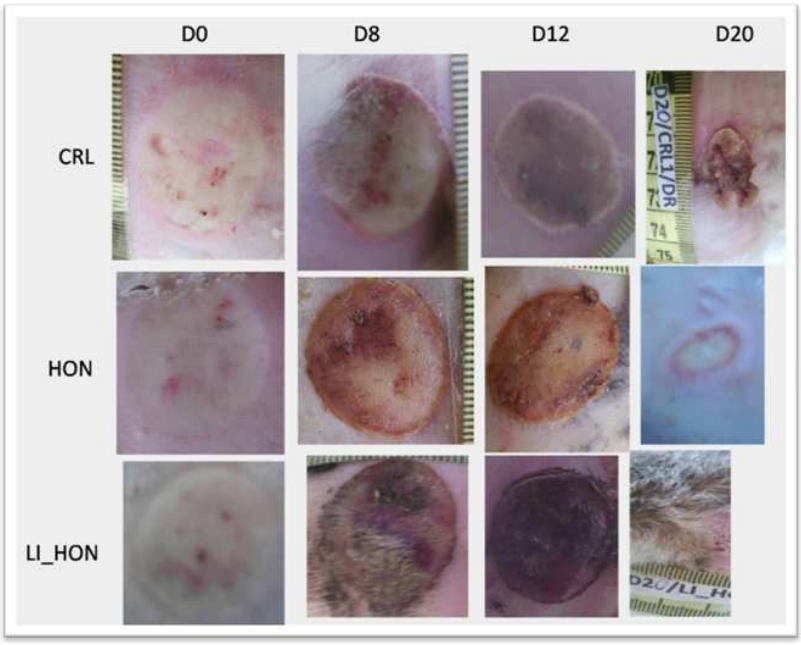

Fig. 3. Photographs of some skin wounds of control group, honey group and *Lawsonia inermis* + honey group at 0, 8, 12 and 20 days after burns. D: Day, CRL: Control (untreated animals), HON: Honey group, LI_HON: *Lawsonial inermis* + honey treated rabbits

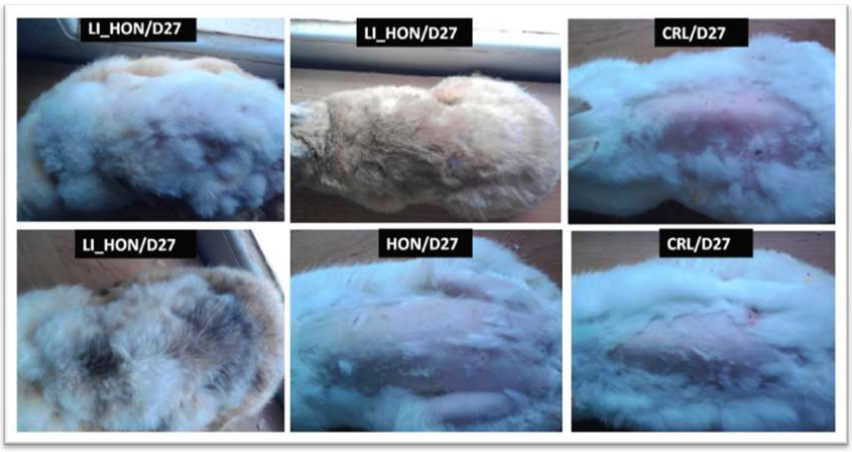

Fig. 4. Photographs of hair growth in the different groups at 27th day post burn

Table 1. Evolution of healing process in control and treated groups

	Necrosis		Start of 1st crust detachment		Complete detachment of 1st crust		Healing time	
	Mean (n = 12)	Var.	Mean (n = 12)	Var.	Mean (n = 12)	Var.	Mean (n = 12)	Var.
CRL	10.75	0.916	12.5	1.66	21,375	17,696	24,5	12
HON	8	0.666	13.5	1.66	19,75	1,583	25	6
LI_HON	6.25	0.916	10.5	0.333	17,25	1,583	19,25	1,583
Statistical data (*P value)*								
HON Vs CRL	0,004		0,315		0,476		0,803	
LI_HON Vs CRL	5,599 E^{-4}		0,030		0,089		0,0165	
LI_Hon Vs HON	0,031		0,005		0,030		0,005	

CRL: Control (untreated animals), HON: Honey group, LI_HON: *Lawsonial inermis* + honey treated rabbits

Table 2. Hair growth at 35th day post burns in control and treated groups

	Percentage of recovered area		New hair length/Normal hair length	
	Mean	Var.	Mean	Var.
CRL	12.33	6.33	0,683	0,010
HON	2.33	2.33	0,75	0,002
LI_HON	98.33	2.33	0,98	4E-4
Statistical data (*P value)*				
HON Vs CRL	0,00417		0,3739	
LI_HON Vs CRL	$9{,}13038E^{-7}$		0,00835	
LI_Hon Vs HON	$1{,}70745E^{-7}$		0,00178	

CRL: Control (untreated animals), HON: Honey group, LI_HON: *Lawsonial inermis* + honey treated rabbits

Discussion

Healing Process

The effect of honey applied alone was significant compared to control in the first 10 days of healing process with no infection of wounds. This period correspond to hemostasis and inflammatory phases (through 4 to 6 days post burns) and a part of the proliferative phase (which prolonged until 14th day) (Broughton *et al.*, 2006). This result could be a consequence of honey pharmacological properties demonstrated in several studies. According to Namias (2003), honey has anti-inflammatory effect, promotes granulation tissue formation and exert antibacterial activity. The anti-inflammatory property may be associated with the antioxidant content of honey (Tanaka *et al.*, 1995). The high osmolarity of honey has been considered a valuable tool in the management of sloughy and septic wounds; it produces a cleansing effect and naturally debrides non-viable tissue. A reduction but not significantly of time of first crusts detachment was recorded in honey group compared to control; this later has recorded a better healing time but not significantly than that of honey. Several studies have explained the effects of honey on granulation tissue formation and epithelialization by the generation of hydrogen peroxide (stimulation of angiogenesis), the growth of fibroblasts and the wound acidification. The nutrient content of honey may also stimulate growth because it has a wide range of amino acids, vitamins and trace elements (Reviewed by Molan, 1999). The addition of *L. inermis* on honey in the third group has resulted in very significant results in the different stages of wound healing. Phytochemical screening of henna plant has revealed the presence of numerous chemicals including alkaloids, tannins, flavonoids, steroids, glycosides, saponins etc. (Ibrahim *et al.*, 2008; Arun *et al.*, 2010). According to Muhammad and Muhammad (2005), the plant constituent are made up of mannite, tannic acid, mucilage and gallic acid, but the main constituent is 2-hydroxynapthoquinone (lawsone), known to be the major bioactive constituent of this plant. In an *in vitro* study, the henna leaves extracts were able to inhibit the growth pattern of *Aspergillus niger* and *Fusarium oxysporum*, *Streptococcus sp*. and *Staphylococcus aureus* which are the primary invaders of burnt wounds. Inhibition of these microorganisms' growth suggests that henna may be valuable in the management of burnt wound infections (Muhammad and Muhammad, 2005). Chloroform extract of leaves of *L. inemris* had shown a highest antioxidant activity compared to α-tocopherol (Endrini *et al.*, 2007). In addition, *L. inermis* L. extract exhibited absolute toxicity and showed broad fungitoxic spectrum when tested against 13 ring worm fungi (Singh and Pandey, 1989). A significant analgesic and antipyretic activities were also shown with leaves extract of this plant (Mohsin *et al.*, 1989). In another study, isoplumbagin and lawsaritol, isolated from stem bark and root of *L. inermis* L. showed anti-inflammatory activity against Carrageenan induced paw edema in rats (Gupta *et al.*, 1993). In a study of Nayak *et al.* (2007), the healing activity of *L. inermis* extract was compared with the control and reference standard animals; the results showed a positive effects on term of wound contraction, epithelialization period, skin breaking strength, granulation tissue weight and hydroxyproline content in this plant group. The authors have conducted a histological study which has showed increased and well organized bands of collagen, more fibroblasts and few inflammatory cells in henna group.

Hair Growth

Honey group has recorded a significant delay in hair growth, compared to control in term of percentage of recovered area, but no statistical significance was observed in comparing new hair length to normal hair length; this later may be due to individual characteristics and/or due to stress because these animals were manipulated more than the untreated animals. The real hair growth promoting activity recorded in LI_HON group is due to *Lawsonia inermis* powder that spreads, after it's sprinkling on the honey, in the whole surface of the shaved back. *L. inermis* has been cited as a growth accelerator and was used in an ancient Egyptian formula to cure the loss of hair. Henna has also been recognized to act as a very good conditioner to the hair

(Ahmadian and Fakhree, 2009). Several histological and molecular studies have focused regeneration of skin and hair follicles after wounding. Ansell *et al.* (2011) have demonstrated an association between hair follicle cycling and wound healing. Chen *et al.* (2015) have shown that collagen VI is strongly deposited in hair follicles and is markedly up regulated by skin wounding; they have highlighted the essential relationships between extracellular matrix and hair follicle regeneration and suggested that collagen VI could be a potential therapeutic target for hair loss and other skin related diseases. These above described mechanisms of influence of the different hair-cycle stages on skin wound healing may be implicated in the interpretation of the present study results.

Conclusion

The mixture of *Lawsonia inermis* L. powder and honey stimulates burn wound healing process in its different stages. In addition to this, henna promotes hair growth. Further studies, including immunohistochemical investigations are required to confirm and elucidate the mechanism behind this promoting hair growth activity.

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Author's Contributions

Zouhir Djerrou: The first author designed and supervised the study and assisted in data analysis and manuscript preparation.

Imane Mokhbi: Sample collection and laboratory experiments.

Khadidja Saci Hadef: Sample collection and participated in laboratory experiments.

Noudjoud Boutobza, Saida Bouzeguine, Ilhem Brighet and Besma Khelfa: Participated in laboratory experiments.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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