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Wound healing effect of a Unani formulation *Marham-e-Ral* in albino rats

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ABSTRACT

Objective: The aim of the present study was to verify the wound healing claims of a Unani formulation *Marham-e-Ral* on different experimental models of wounds in albino rats.
Material and methods: *Marham-e-Ral* was prepared as per classical method. The animals were divided into three groups of six each. The parameters studied were wound contraction, epithelialisation period and tensile strength.
Results: *Marham-e-Ral* showed wound healing activity, with almost 98.9% healing on 15th day, whereas in the control only 90% healing took place. In the incision wound studies, there was a significant increase in tensile strength on day 10 due to treatment with standard cream (31.90 ± 0.026) and *Marham-e-Ral* (16.48 ± 0.022) when compared with control group i.e. 8.388 ± 0.128 ($P \leq 0.01$). The histopathological slides clearly showed the extent of healing and demonstrated significant improvement.
Conclusion: *Marham-e-Ral* showed a wholesome effect on wound healing in excision wound model and the effect was found equally comparable to the standard drug framycetin cream.

INTRODUCTION

One of the most important compound formulations of Unani system is *Marham-e-Ral*. It is used as wound healer and also effective in various skin lesions like boils, syphilitic ulcer and fistula, etc. (Khan, 1996; Said, 1997; Anonymous, 1986).

The literature survey revealed that the ingredients of *Marham-e-Ral* contain triterpenoids and chalcone glycoside in *Shorea robusta* (Anonymous, 1995; Jain et al., 1982), glycerides and flavonols in *Cinnamomum camphora* (Kafoor) (Mukherjee et al., 1994), catechin, tannins, glycoside and quercetin in *Acacia catechu* (Katha), (Azad, 2001; Trease and Evans, 2002; Yadav & Sodhi, 2002), esters and alcohols in Beeswax (Jackson, 2006; Tulloch, 1971), and volatile acids & glycerides in Ghee (Prasad & Dorle, 2006). Several pharmacological studies of *Marham-e-Ral* have been reported *viz*. *Shorea robusta* posses anti-inflammatory and anti-bacterial activity (Das et al., 2003). *Cinnamomum camphora* has anti-inflammatory action due to modulation of cytokine, NO & prostaglandin E_2 (Lee et al., 2006) and antifungal activity (Srivastava et al., 2008). *Acacia catechu* showed antioxidant (Naik et al., 2005) and antimicrobial activity (Singh et al., 2005). Beeswax posses antiulcer activity (Carbajal, 1995) and Ghee has wound healing activity (Charde et al., 2006; Prasad & Dorle, 2006). On the basis of above mentioned references *Marham-e-Ral* was chosen for the evaluation of wound healing potential in rat models.

MATERIAL AND METHODS

Marham-e-Ral

Ingredients of *Marham-e-Ral* are as follows: Ral (Oleo Resin of *Shorea robusta*)-50 g, Kafoor (Solid ketone of *Cinnamomum camphora*)-50 g, Katha (Extract of *Acacia catechu*)-50 g, Roghan-e-Gao (Clarified butter of cow)-200 g, Wax (Bees wax)-50 g, (Said, 1997; Anonymous, 1986).

Plant materials

Ral (Oleo Resin of *Shorea robusta*), Kafoor (Solid ketone of *Cinnamomum camphora*) and Katha (Extract of *Acacia catechu*) were purchased from Green Earth Pvt. Ltd., New Delhi, India. Beeswax was purchased from Khari Bawli, Delhi, India and Ghee was obtained from Amul outlet, New Delhi, India. The authenticity and identity of the drugs were confirmed on the basis of classical description in Unani literature at Department of Ilmul Advia, Faculty of Medicine (Unani) and modern botanical information was established by Department of Botany, Faculty of Science, Jamia Hamdard, New Delhi, India. Standard used was Framycetin sulphate 1% cream (Aventis) (Manjunatha, et al., 2005).

Preparation of *Marham-e-Ral*

Marham-e-Ral was prepared as per classical method described in Unani literature (Said, 1997; Khan, 1996; Anonymous, 1993; Anonymous, 1986). Ral (*Shorea robusta*), and Katha (*Acacia catechu*) were finely ground and sieved through 100 number mesh after which the camphor was first triturated with little and later the whole safoof. The ghee and wax were melted and powdered mixture of katha, ral & kafoor was added to the ghee-wax mixture, stirred till both mixed well and stocked in a glass jar.

Experimental animal

Healthy male/female inbred albino rats of Wistar strain of 4-6 months; weighing 150-200 g were used in the present study. They were procured from Central Animal House Facility, Jamia Hamdard and were housed and maintained at standard housing. They were fed with a commercial diet and water *ad libitum* during experiment. Approval for experimental studies was obtained from Institutional Animal Ethical Committee, Jamia Hamdard, Hamdard Nagar, New Delhi, India.

Excision wound model

The rats were fasted overnight and were inflicted with excision wounds (Morton & Malone, 1972). Animals were anaesthetized by open mask method with anesthetic ether and their back were shaved with depilatory and cleaned with dry gauze piece. Rats were depilated on the back and a circular wound of about 500 mm^2 full thickness were excised in the dorsal inter scapular region (Mukherjee et al., 2000). Full aseptic measures had not been taken and no local or systemic antimicrobials were used throughout the experiment. The animals were divided into three groups (control, standard and *Marham-e-Ral*) of six animals each. The creams were topically applied once a day, starting from the day of operation, till complete epithelialization. The parameters studied were wound contraction, epithelialization period.

Measurement of contraction and epithelialization of wound

Contraction which mainly contributes for wound closure was studied by tracing the raw wound area on transparent paper on day 3, 6, 9, 12, and 15th and thereafter every alternate day till wounds were completely covered with epithelium (Manjunatha et al., 2005). These wound tracing were retraced on a millimeter scale graph paper to determine the wound area. On each measurement day the wounds of the animals were photographically documented. Wound contraction (WC) was calculated as a percentage change in the initial wound size i.e.

$$WC(\%) = \frac{\text{Initial wound size–specific day wound size}}{\text{Initial wound size}} \times 100$$

Epithelialization period was monitored by noting the number of days required for eschar to fall away, leaving no raw wound area behind (Kamath, et al., 2006).

Incision wound model

The animals were anesthetized with intra peritoneal injection of pentobarbitone in the dose of 50 mg/kg; and their back were shaved with depilatory and cleaned with dry gauze piece, and a 6 cm long para-vertebral incision of the skin on either side of the vertebral column of the rat was made (Ehrlich et al., 1968). The wounds were made 1 cm away laterally to the vertebral column, after hemostasis, wounds were cleaned with sterile wet cotton and then closed with interrupted sutures of 1cm apart using a surgical thread (no. 000) and a curved needle (no. 11) (Swamy et al., 2007; Lodhi et al., 2006; Singh et al., 2006). Immediately after operation, the rats were placed in the collars to prevent damage to the wounds. Full aseptic measures had not been taken and no local or systemic antimicrobials were used throughout the experiment. The animals were divided into 3 groups (control, standard and *Marham-e-Ral*) of 6 animals each. The creams were topically applied once in a day. On the 9th day after wounding sutures were removed and the tensile strength was measured on the 10th day (Mukherjee et al., 2000; Singh et al., 2006).

Measurement of tensile strength of the wound

Tensile strength is the resistance to breaking under tension. It indicates how much the repaired tissue resists to breaking under tension and may indicate in part the quality of the repaired tissue. For this purpose the newly repaired tissue including scar was excised to measure the tensile strength (Rashed et al., 2003). Tensile strength of wounds was determined in all three groups on day 10 by texture analyzer (TA XT2). The rats were anesthetized with light ether. The skin was removed with 1 cm on each side of the wound. Tensile strength was measured using a texture analyzer (TA XT2) and the increase in tensile strength served as a measure of wound healing. Tensile strength was determined using the following equations (Saringat & Sheikh, 2000).

Tensile strength = Breaking load (force)/Area

Area = Thickness × Width

Statistical analysis

All the values were expressed as mean ± SEM. The statistical significance was determined by one-way ANOVA followed by Dunnett test. Values $P < 0.05$ and $P < 0.01$ were considered as significant and $P < 0.001$ as highly significant.

RESULTS

Excision wound test

The mean percentage closure of wound area was calculated on 3rd, 6th, 9th, 12th, 15th and 18th post wounding day. The observations are shown in the table 1 & 2 and fig. 1-8. In control group wound contracted to the extent of 90.3% by day 15 on the other hand *Marham-e-Ral* showed wound contraction 98.93% which is significant ($P < 0.01$), to control group. The period of epithelialization of wounds were found to be faster i.e. 16.00 ± 0.36 day in *Marham-e-Ral* group as compared to the animals of control group where it was found

Table 1. Effect of *Marham-e-Ral* on post wounding reduction in wound area in excision wound model.

Post Wound Days	Post wounding reduction in wound area in cm^2		
	Control	**Standard**	***Marham-e-Ral***
Day 0	4.98 ± 0.007	4.971± 0.006	4.98 ± 0.005
Day 3	4.351± 0.009	3.923 ± 0.038**	3.90 ± 0.044**
Day 6	3.586 ± 0.019	2.966 ± 0.008**	2.968 ± 0.037**
Day 9	2.04 ± 0.005	1.37 ± 0.005**	1.575 ± 0.014**
Day 12	0.951 ± 0.007	0.27 ± 0.003**	0.341 ± 0.007**
Day 15	0.466 ± 0.006	0.047 ± 0.01**	0.053 ± 0.002**
Day 18	0.126 ± 0.003	0.001 ± 0.000**	0.002 ± 0.002**
Day 21	0.025 ± 0.002	0.000	0.000

The groups were compared by one-way ANOVA followed by Dunnett test.

Values are expressed as mean ± SEM, n = 6.

*P < 0.05, **P ≤ 0.01 vs. control

21.33±0.21 day. While period of epithelialization was 15.33 ± 0.21 day in the standard group. The differences in mean epithelialization of wounds treated by *Marham-e-Ral* and compared with control groups was found significant (P < 0.01).

Incision wound test

In incision wound model, the mean breaking strength of 10 days old wound was significantly increased in the animal group treated with standard drug framycetin 1% w/w cream i.e. 31.90 ± 0.026 n/cm^2 (P < 0.01). The mean breaking strength in *Marham-e-Ral* group was measured as 16.48 ± 0.022 (P < 0.01) and was found significant (P < 0.01), when compared with the control group (8.388 ± 0.128). The observations are shown in the Table 3.

Table 2. Effect of test formulations on wound contraction & period of epithelialization in excision wound model.

Groups	Percentage (%) wound contraction in days							Period of epithelialisation
	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Days
Control	0.00	12.6	27.99	59.03	80.9	90.64	97.46	21.33 ± 0.21*
Standard	0.00	21.08	40.33	72.44	94.56	99.05	99.97	15.33 ± 0.21**
Marham-e-Ral	0.00	21.68	40.4	68.37	93.15	98.93	99.95	16.0 ± 0.36**

The groups were compared by one-way ANOVA followed by Dunnett test.

Values are expressed as mean ± SEM, n = 6.

*P < 0.05, **P ≤ 0.01 vs. control.

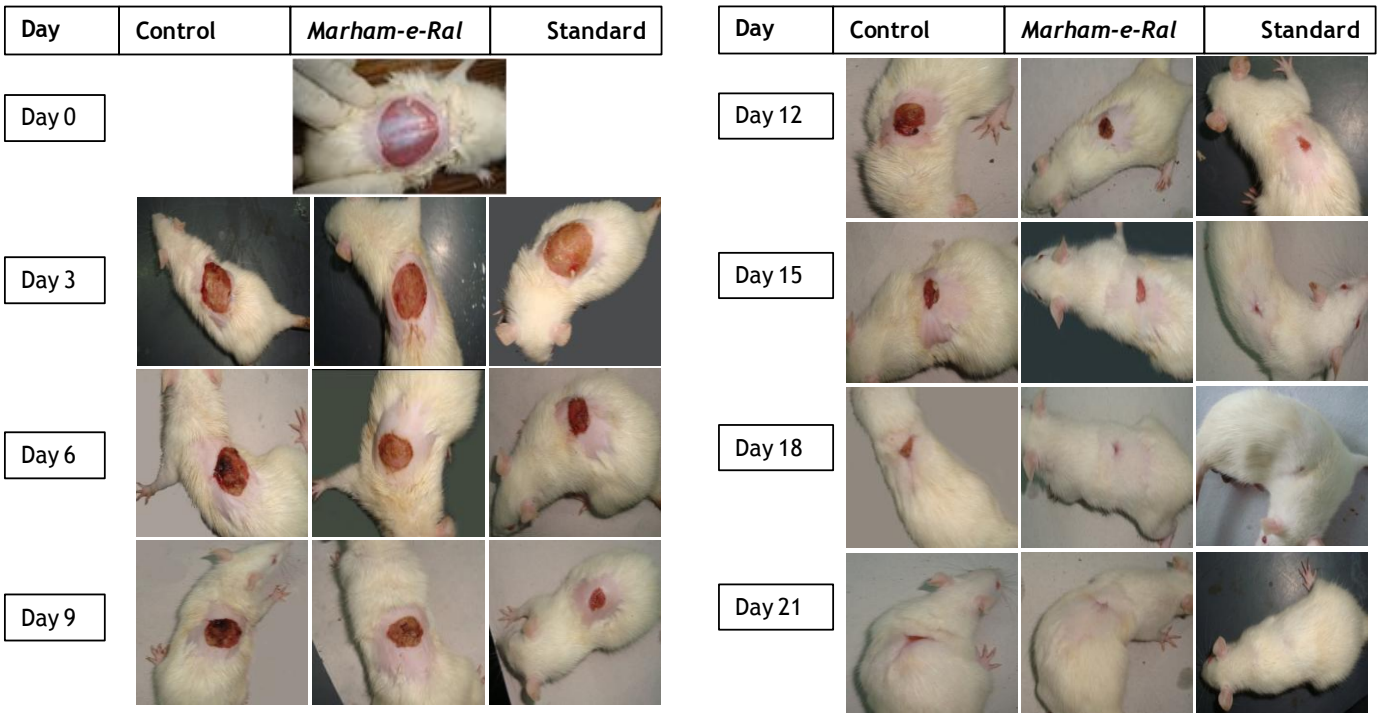

Figure 1. Photographic representation of wound contraction on various post-excision days (0–21 days).

Table 3. Effect of test formulations on breaking strength in incision wound model.

Formulations	Area of skin (cm^2)	Force applied (N)	Tensile strength (N/cm^2)
Control	0.67	5.623 ± 0.086	8.388 ± 0.128*
Standard	0.66	21.063 ± 0.017	31.90 ± 0.026**
Marham-e-Ral	0.67	11.045 ± 0.014	16.48 ± 0.022**

The groups were compared by one-way ANOVA followed by Dunnett test.

Values are expressed as mean ± SEM, n = 6.

*P < 0.05, **P ≤ 0.01 vs. control.

DISCUSSION

Herbal medicines in wound management involve disinfection, debridement and providing a moist environment to encourage the establishment of the suitable environment for natural healing process (Priya et al., 2002). The results in table 1-3 indicate that the *Marham-e-Ral* have wound healing activity, with 98.9% healing on 15th day, similar to standard (99.05%), where as in the control only 90 % healing took place on 15th day. The effects produced by test ointment were

comparatively lesser than standard but produced better response in comparison to control group. The literature survey revealed that the ingredients of *Marham-e-Ral* has been screened scientifically for its efficiency by various researchers and has been reported to contain a number of phytochemicals, which are responsible for its various important pharmacological actions (Mukherjee et al., 1994; Azad, 2001; Trease and Evans, 2002; Yadav & Sodhi, 2002).

The present study found the anti-inflammatory activity of *Marham-e-Ral* in Wistar albino rats, representing different phases of inflammation. Wound healing is stepwise process, which comprises of various phases such as hemostasis, inflammation, proliferative and remodeling or maturation. The genetic reaction regulating the body's own cellular resistance mechanisms plays to the wound and its repair (Charles et al., 1995). Therefore, in the present study, excision and incision wound models were kept to assess the effect of *Marham-e-Ral* on different sphases. In incision wound, enhance intensile strength of treated wounds may be because of enhance in collagen concentration and stabilization of the fibers (Udupa et al., 1995).

The results showed that *Marham-e-Ral* possesses a marked prohealing stroke demonstrated by a significant enhance in the rate of wound contraction and by increased epithelialization period. Recent studies with other plant extracts have revealed that phytochemical constituents like flavanoids (Tsuchiya et al., 1996), triterpenoids (Scortichini et al., 1991) and tannins (Rane et al., 2003) are known to encourage the wound-healing process. Preliminary phytochemical screening constituents of *Marham-e-Ral* exhibited the presence of alkaloids, flavonoids and tannins (Mukherjee et al., 1994; Azad, 2001; Trease and Evans, 2002; Yadav & Sodhi, 2002). Presence of these ingredients may have promoted the wound healing process mainly due to their astringent and antimicrobial property, which seems to be responsible for wound contraction and increased rate of epithelialisation. The analgesic and anti-inflammatory effects prove to be of added advantage in such conditions.

CONCLUSION

It can be concluded that the *Marham-e-Ral* is able to potentiate the natural healing process, reducing the time period of onset of healing and the completion of healing process until the epithelialisation took place. It is obvious that pain and inflammation will be relieved earlier than the natural healing time.

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CONFLICT OF INTEREST

None declared.

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