

EVALUATION OF WOUND HEALING ACTIVITY WITH A NEW FORMULATION OF DRY *MANGIFERA INDICA* AND HONEY USING SWISS ALBINO MICE

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ABSTRACT

Objective: A new formulation was made with dry *M. indica* power in combination with honey to understand the activity of wound healing on Swiss albino mice.

Methods: Initially, animals were grouped into four groups, and a wound of 150 mm² was created. All the groups were taken for the study, and wound contraction was studied on different days. The control group was treated with saline, Vitamin E was used as standard of the care, and other groups were treated with 200 mg/kg and 500 mg/kg of the new formulation.

Results: Wound healing started after 4th day in the standard treated group, and the other formulation treated groups also started wound contraction from 2nd day onward, whereas control did not show any wound contraction after 2nd day. However, significant wound contraction observed after treatment with 500 mg of formulation on the 10th day, and this cannot be compared with the standard group on the 12th day. Even good wound contraction observed with 200 mg/kg of the formulation which is similar to that of standard treated groups. The control group showed wound contraction of about 34.72 mm², whereas the standard group showed 9.58 mm² and the formulation-treated groups 200 mg/kg and 500 mg/kg showed 15.39 mm² and 8.48 mm².

Conclusion: This observation showed that a good wound contraction observed with a new formulation.

Keywords: *Mangifera indica*, Honey, Wound healing, Wound contraction, Swiss albino mice.

INTRODUCTION

Mangifera indica fruit contains mangiferin, which is a polyphenolic antioxidant and a glucosyl xanthone, and having a strong antioxidant, antilipid peroxidation, hypotensive, wound healing, antidegenerative, immunomodulation, cardiotoxic, and antidiabetic activities [1]. They are useful in dysentery ophthalmia, eruptions, urethrorrhea, and vaginopathy [2]. The active constituents of the plant show anti hyperglycemic effect [3]. It is also used for anti-inflammatory, analgesic, and hypoglycemic properties [4]. The plant products proved to be effective against antitumor, immunomodulatory, and anti-HIV properties [5]. The chemical constituents of the plant *M. indica* contain especially the polyphenolics, flavonoids, and triterpenoids. Mangiferin a xanthone C-glycoside major bio-active constituent, isomangiferin, tannins, and Gallic acid derivatives. The fruit pulp contains Vitamins A and C, β -carotene, and xanthophylls. An unusual fatty acid, cis-9 and cis-15-octadecadienoic acid, was isolated from the pulp lipids of mango [6]. These are all the active constituents responsible for various activities. Mangiferin probably shows wound healing activity with the presence of Vitamin A and C, which are responsible for the formation of collagen synthesis. The honey produced by honey bees with genus *Apis* is one of most commonly used antimicrobial agents, which are essentially required for treating various infections. However, when any resistant to pathogens develops the usage of antibiotics will not be suitable. This has become a serious problem to the public health, and there is a need to develop new antibiotic combinations for various infections [7,8]. That's the reason there is always need to innovate new antimicrobial agents which can show good therapeutic activity [9-11]. Now, so many studies reported about honey which showed profound antibacterial activity where natural unheated honey has some broad-spectrum antibacterial activity when tested against pathogenic bacteria, oral bacteria as well as food spoilage bacteria [12,13]. The honey, when applied topically, rapidly clears wound infection to facilitate healing of deep surgical wounds with infection [14]. The application of honey can promote the

healing in infected wounds that do not respond to the conventional therapy, i.e., antibiotics and antiseptics including wounds infected with methicillin-resistant *Staphylococcus aureus* [15,16]. Mangiferin in combination with honey not made a formulation so far for wound healing activity, since honey is having so many medicinal properties, made with combination and tried to heal the wounds.

METHODS

Selection of animals

Healthy mice of Swiss albino strain of male sex, weighing 25-30 g, were selected for the study. The experimental protocol was approved by the Institutional Animal Ethics Committee, and the whole experimentation was done under CPCSEA guidelines (IAEC/006/NCPA/B.Pharm/2015-2016).

Maintenance of animals

The animals were kept in the animal house with good ventilation and maintained at 20-24°C for one week to acclimatize the conditions before experimentation. Animals were provided with standard rodent pellets and purified water. Since all the wounded animals were susceptible to infection, hygienic conditions were maintained.

Drug material

Big sized, unripe, green mango fruits obtained locally. The fruits were peeled off and the seeds were removed and the fruit part was cut into small pieces, dried under room temperature, powdered and stored for further use. Honey was also collected from honeycomb and stored.

Standard drug

Vitamin E (200 mg/kg) was taken as a standard drug for comparison of wound healing activity on animals. Vitamin E of required weight (200 mg/kg) was calculated, weighed, and applied to standard group animals.

Table 1: Measurement of wound diameter of various groups of animals

S.no	Groups	Wound contraction in mm ²					
		0 day	2 day	4 day	6 day	8 day	10 day
1	Control	145.42±5.19	116.94±2.35	85.09±3.06	34.72±4.36	11.78±2.32	5.50±0.96
2	Standard Vitamin E (200 mg/kg)	145.42±5.19	81.8±1.96	41.16±4.16	9.58±1.93	2.63±0.51	0.35±0.14
3	Test (200 mg/kg)	141.18±5.19	86.77±3.67	55.64±3.29	15.39±1.73	4.71±0.96	0.47±0.12
4	Test (500 mg/kg)	145.42±5.19	77.22±3.72	38.80±3.48	8.48±1.81	1.61±0.62	0.00

Table 2: Percentage inhibition of wound contraction

Groups	Percentage inhibition of wound contraction							
	0 day	2 day	4 day	6 day	8 day	10 day	12 day	14 day
Control	0	19.58	41.49	76.12	91.90	96.2	99.84	100.00
Standard Vitamin E (200 mg/kg)	0	43.75	71.70	93.41	98.19	99.0	100.00	100.00
Test (200 mg/kg)	0	38.54	60.59	89.10	96.66	98.0	100.00	100.00
Test (500 mg/kg)	0	46.90	73.32	94.17	98.89	100.00	100.00	100.00

Preparation of formulation

The weight of dry *M. indica* powder required for the treatment was calculated based on the body weights of the animals. The required quantity of dry *M. indica* was weighed and was mixed in honey by triturating to get mixed in the honey. All the doses were prepared after preparation of formulation.

Experimental design

The animals were weighed and divided into four groups with 5 animals in each group as follows:

- Group I: Control (not treated with any drug)
- Group II: Treated with standard Vitamin E ointment
- Group III: Treated with test drug formulation (200 mg/kg)
- Group IV: Treated with test drug formulation (500 mg/kg).

Animals were anesthetized with diethyl ether during wound creation. At the predetermined area of wound, the dorsal fur of the animal was shaved with electric trimmer and the circular area of the wound was marked. The excision wound, a circular piece of full thickness sized approximately 150 mm² and 1 mm depth was made by cutting along the markings using pointed forceps and pointed scissors from the shaved area. (Fig. 3) The entire wound was left open and then the treatment was started (As stated above). Wound contraction was calculated as percentage reduction in wound.

$$\% \text{Wound contraction} = \frac{\text{Healed area}}{\text{Total wound area}} \times 100$$

(Healed area = original wound area present wound area)

The rate of wound contraction was assessed by tracing the wound on day 0, 2, 6, 8, 10, 12, and 14 using plastic sheet and marker.

Statistical analysis

The experimental results were expressed as multiple comparisons of mean±standard error of the mean, the results statistical significance obtained by two-way analysis of variance followed by Dunnett multiple comparisons test, and statistical significance was defined as p<0.05.

RESULTS

The study of wound contraction of the new formulation of dry *M. indica* and honey had shown a significant wound contraction in all the treated groups. Wound healing started after 4th day in the standard treated group and the formulation-treated groups, started wound contraction from 2nd day onward only, whereas control did not show any wound contraction after 2nd day. While 500 mg/kg treated, group had healed their wounds completely within 10th day of the treatment where

standard group showed complete healing on the 12th day (Table 1 and Fig. 1). In the groups of 200 mg/kg new formulation and the standard treated group, there was a good and similar contraction observed. On the 10th day of treatment, the new formulation of 500 mg/kg treated group had shown 100% wound contraction, whereas on the 12th day of treatment 200 mg/kg of new formulation treated group and standard Vitamin E (200 mg/kg) treated group had shown 100% contraction. (Table 2 and Fig. 2) While the control group which was treated with saline had shown 100% wound contraction on the 14th day of treatment. From this above information, the new formulation had shown a good wound healing property.

DISCUSSION

The biggest problem of applying antibiotic ointments is it may lead swelling and an allergic reaction called contact dermatitis [17]. To achieve these problems, always herbal formulations are better for wound healing activity. There were so many plant products; formulations were used for wound healing; now for the better and faster wound healing process, we have taken honey - an antibacterial agent combined with *M. indica* fruit powder which is rich of Vitamin C and A helps in faster wound healing. These two were made into formulation and applied on wounds of the animals. Honey has already proven to have an inhibitory effect on around 60 species of bacteria including aerobes and anaerobes, Gram-positives and Gram-negatives [18,19]. The honey has been used from ancient times as a method of accelerating wound healing [17], and the potential of honey to assist with wound healing has been demonstrated repeatedly [20,21]. Honey is gaining acceptance as an agent for the treatment of ulcers, bed sores, and other skin infections resulting from burns and wounds [22,23]. There are many reports of honey being very effective as dressing of wounds, burns, skin ulcers, and inflammations; the antibacterial properties of honey speed up the growth of new tissue to heal the wound [24]. However, for the fastest wound healing process, it was formulated with *M. indica* fruit powder and applied on the wounds. *M. indica* fruit powder contains several nutritional factors required for wound repair may improve healing time and wound outcome [25]. Vitamin A helps in epithelial and bone formation, and it improves the function of immune system and also in cellular differentiation [26]. Vitamin C is required for collagen formation and improves immunity, proteoglycans, and other organic components of the intracellular matrix of tissues such as bones, skin, capillary walls, and other connective tissues [27]. Vitamin E is the better lipid-soluble antioxidant in the skin it inhibits peroxidation of lipids and leads to the formation of more stable cell membranes. The antioxidant-membrane stabilizing effect of Vitamin E includes lysosomal membrane stabilization, a function of glucocorticoids. Systemic Vitamin E and glucocorticoids inhibit the inflammatory response and collagen synthesis [27,28]. Based on these properties, we have chosen this vitamin as a standard. When we formulated both Honey and *M. indica*, they showed good wound healing

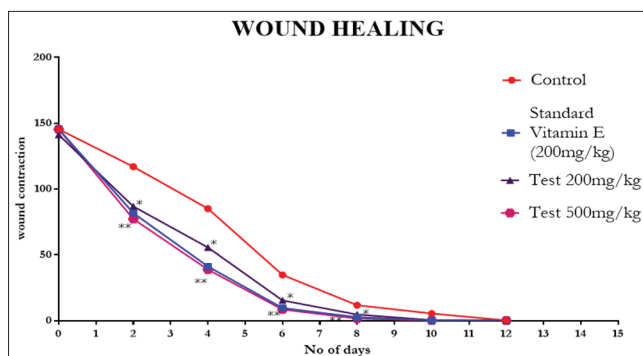


Fig. 1: Wound contraction observed at various days

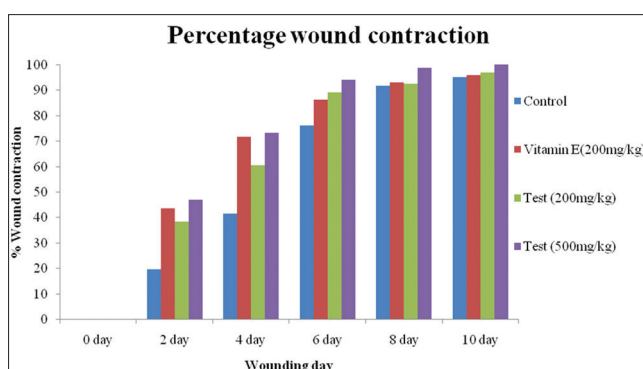


Fig. 2: Percentage wound contraction

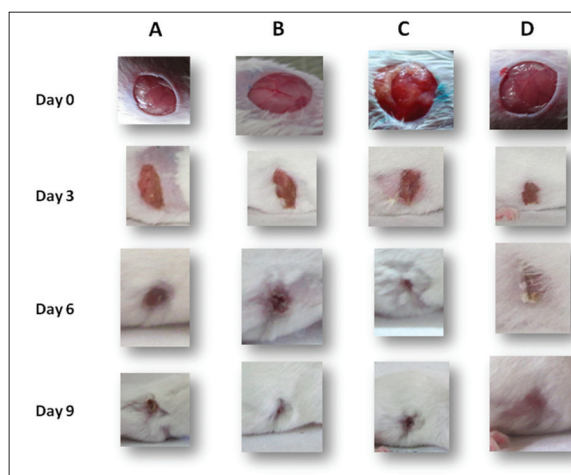


Fig. 3: The process of wound healing observed in mice from day 0 to day (a) control (b) standard Vitamin E (c) test formulation (200 mg/kg) (d) test formulation (500 mg/kg)

activity by 10th day, whereas the control group showed after 14th day. This trend did not continue with the standard treated group where it showed only after 10th day. This study shows good wound healing activity can be achieved when formulated these two compounds together other than using these separately.

CONCLUSION

From the present study, it is clear that the formulation of honey and *M. indica* fruit powder and honey showed good wound healing activity, but the exact mechanism of action was not found out. However, when we further study by taking the tissue samples at the site of wound and understand the repairing mechanism only, we can come to know the mechanism of wound healing.

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