Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2013, 5 (5):256-264
(http://scholarsresearchlibrary.com/archive.html)

Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Pharmacological Evaluation of Honey, Daruhaldi and Shatdhaut ghrut on wound healing activity in excision model in rats

Swati A. Moholkar* and P. S. Worlikar

Department of Pharmacology, Padmashree Dr. D. Y. Patil Medical College & Dr. D. Y. Patil University, Pimpri, Pune , Maharashtra, India

---

ABSTRACT

The aim of present investigation was to study the therapeutic potential of Honey, Daruhaldi and Shatdhaut ghrut and honey singly and in combination on experimental excision wound healing activity in rats. Excision wounds of about 500 mm^2 and 2 mm depth were used for the study. Parameters studied were period of epithelization, rate of wound contraction and time required for 50% wound closure (CT-50). Rats treated with Honey + Daruhaldi combination showed better wound healing activity as compared to other test drugs. Effect of this combination on wound area was less as compared to povidone iodine. However effect of Honey + Daruhaldi & Povidone iodine were comparable in % wound contracture or % wound closure. In this Excision wound model shatdhaut Ghrut alone or in combination with Honey showed less wound healing activity as compared to povidone iodine. The improved wound healing potential and synergistic effect of Honey & Daruhaldi can be attributed to additive antioxidant potential of Honey and flavonoid moities in Daruhaldi combination showed better wound healing potential than Honey & Daruhaldi alone.

Keywords: Excision wound, Daruhaldi, Honey, Shatdhaut ghrut, wound area.

INTRODUCTION

Wounds are indispensible part of human life and agents like pathogens, damaged cells or irritants induced wounds [1]. The loss or breaking of cellular and anatomic or functional integrity of living tissues is generally termed as wound [2]. In the developing country due to poor hygienic conditions the wound is a serious debilitating problem [3]. There are different types of wounds which range from mild to potentially fatal. Throughout the world about 6 million people are encumbered with chronic wounds. According to Mazumdar & Mukhopadhay prevalence of acute and chronic wound is 10.5 per 1000 population & 4.5 per 1000 population respectively [4].

Healing is generation and maintenance of normal anatomical cellular structure and function for the purpose of the survival. Generation is preliminary step whereas it ends with formation of scar [5]. Haemostasis, inflammation, proliferation and finally remodeling are the four stages of wound healing process [6]. According to Diegelmann and Evans, healing of acute wound does not require much attention but still wounds are more prone to infection which cause discomfort hence, assistance of rapid wound healing agents is required [7, 8].

Excision wound model is well established, reproducible animal model which is used regularly in preclinical study of drugs in wound healing [9-11].

Research on wound healing drugs is a developing area in modern biomedical science. Ayurveda is a traditional system of medicine. Synthetic chemical moieties are present in the treatment regimens for the management of under healing of wound, however about 80% of the population still depends upon Ayurveda for their treatment of diseases [12]. In the allopathic medicine the treatments available for wounds are supportive. Modern drugs act by preventing infection but they do not promote healing process. There are certain products available in the market for example Platelet derived growth factor (PDGF) which stimulates the process of wound healing but these products are expensive and their cost limits their use [13-14]. Therefore there is need to find products which show good wound healing activity and are cost effective for better wound care.

Daruhaldi, Honey, Shatdhaut ghrut, Triphala, Jestimadh etc. are described in Ayurveda for array of therapeutic potential. Triphala & Jestimadh were studied in our laboratory & showed excellent wound healing properties [15]. Moreover, various authors have reported honey as an effective agent in the healing of wounds and burns [16, 17]. Honey possesses antimicrobial, antiulcer as well as antidiabetic property [18-20]. *Berberis aristata* DC (Berberidaceae) is commonly called as 'Daruhaldi'. Experimental pharmacological studies have shown that the plant possesses diaphoretic, antibacterial, antidiarrhoeal [21, 22], anti-inflammatory and immunostimulating properties [23]. Shata-dhauta-ghrita (SDG) is an Ayurvedic preparation and it is commonly used in treatment of wounds, burns, chicken pox, scars, herpes, leprosy and other skin diseases [24].

In view of the above the present study was planned to study the therapeutic potential of Honey (H), Daruhaldi (DH) and Shatdhaut ghrut (SDG) as well as their combination i.e. Honey + Daruhaldi (H + DH) and Honey + Shatdhaut ghrut (H + SDG) on experimentally induced excision wounds in rats.

MATERIALS AND METHODS

2. Material:

Standard Agmark brand of Honey (H), water based paste of Daruhaldi (DH) and Shatdhaut ghrut (SDG) were purchased from Vatsal Ayurvedic, Nasik.

2.1. Animals:

Healthy adult male Sprague-Dowley rats (150-200 g) were obtained from the D. Y. Patil Medical College, Pune (India). The animals were housed in 7 groups in solid bottom polypropylene cages. They were maintained at $24^0C \pm 1^0C$, with relative humidity of 45-55% and 12:12 h dark/light cycle. The animals were acclimatized for a period of two weeks and were kept under pathogen free conditions. The animals had free access to standard pellet chow (Chakan Oil Mills, Sangli) throughout the study. The animals were provided filtered water. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Padmashree Dr. D. Y. Patil Medical College, Pune.

2.2. Chemicals:

Ketamine manufactured by Aqua Fine Injecta Pvt Ltd., Pune was purchased from local Chemist.

2.3. Excision wound model:

Excision wounds were used for the study. Animals were anaesthetized with 80 mg/kg dose of ketamine (i.p.) and hairs on the back of the animals were depilated by applying hair removal cream. An impression was made on the dorsal thoracic region 1 cm away from vertebral column and 5 cm away from ear of the anaesthetized rat. Excision wounds sized 500 mm^2 and 2 mm depth were made by cutting out layer of skin of the marked area. Haemostasis was achieved by blotting the wound with cotton swab soaked in normal saline [6, 10]. The study comprised of seven different groups of six animals in each group one ml of study drugs was applied topically in all study animals of excision wound:

- **Group I** - Control animals: did not receive any treatment.
- **Group II** - Povidon iodine (PI)
- **Group III** - Honey (H)
- **Group IV**- Daruhaldi (DH)
- **Group V**- Shatdhaut ghrut (SDG)
- **Group VI** - Honey + Daruhaldi (H + DH)
- **Group VII** - Honey + Shatdhaut ghrut (H + SDG)

2.3.1. Measurement of wound area:
The progressive changes in wound area were monitored by a camera (Fuji, S20 Pro, Japan) on predetermined days i.e., 0, 4, 8, 12, 16 and 21. The wound areas were marked on transparency sheets and measured by tracing the wound on a millimeter scale graph paper.

2.3.2. Determination of percent wound contraction:
Wound contraction was calculated as percentage of the reduction in original wound area size [6]. It was calculated by using following formula:

$$\text{Percentage wound contraction} = \frac{\text{Initial area of Wound} - \text{Nth day area of wound}}{\text{Initial area of Wound}} \times 100$$

2.3.3. Determination of CT-50 (50% wound closure):
The graph of (% wound closure) versus (time in days from wound creation) was plotted in software (Graphpad Prism v 5) and linear regression analysis was performed. The value of X at Y=50% was taken as CT-50 (half-closure time, Time taken to close the wound by 50%) (Engelmayer et al., 2008).

2.4. Data and statistical analysis:
All the results were expressed as mean ± S.E.M. Data of wound area on day 21 was analyzed using one-way ANOVA; Dunnett's multiple range test was applied for post hoc analysis. Whereas data of wound area and percent wound contraction was analyzed using two-way repeated ANOVA, Bonferroni's multiple range test was applied for post hoc analysis. Data analysis was performed using GraphPad Prism 5.0 software (GraphPad, San Diego, USA). A value of $p < 0.05$ was considered to be statistically significant.

RESULTS

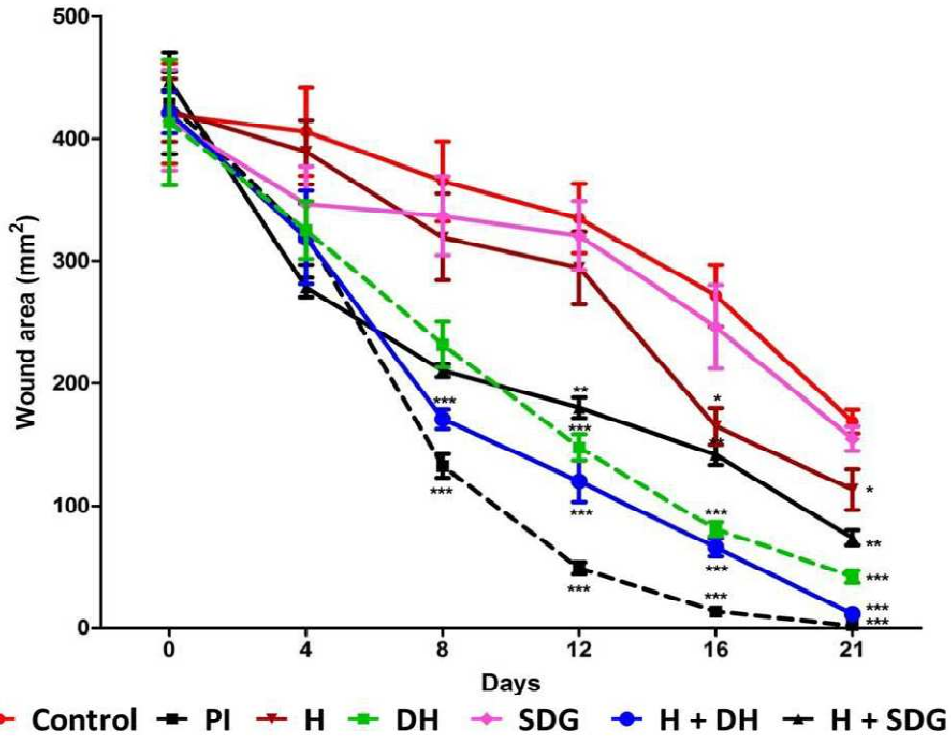

Figure 1 Effect of treatment of honey, Daruhaldi and Shatdhaut ghrut on wound area in rats.
*Data are expressed as mean ±S.E.M. from six rats a nd analyze by two Way ANOVA followed by Bonferroni's test. *P < 0.05, **P < 0.01, ***P < 0.001 as compared to vehicle control group rats*

3.1. Effect of treatment of honey, Daruhaldi and Shatdhaut ghrut on wound area:

On day 0, 4, 8, 12, 16 and 21 the wound area (mm^2) was measured in the all study of animals. The wound area in the control group was 420.50 ± 41.09, 405.83 ± 36.48, 365.16 ± 32.70, 334.83 ± 28.36, 271.50 ± 25.19 and 168.33 ± 9.76 mm2 on respective days. Rats treated with honey showed significant reduction in the wound area on the 16 and 21 days (164.5 ± 14.85 and 113.0 ± 16.52 mm^2, $P < 0.05$) as compared to control group. Whereas treatment with daruhaldi showed significant reduction in wound area on day 12, 16 and 21 (147.33 ± 10.74, 80.5 ± 5.86 and 41.5 ± 5.03 mm^2, $P < 0.001$) as compared to control group. Treatment with shatdhaut ghrut failed to produce significant reduction in the wound area as compared to control group. When compared with control group, rats treated with honey and daruhaldi combination showed significant reduction in the wound area (170.33 ± 8.07, 119.83 ± 16.81, 65.33 ± 7.07 and 11.33 ± 2.66 mm^2, $P < 0.001$) on day 8, 12, 16 and 21. The wound area in honey and shatdhaut ghrut combination treated rats on day 12, 16 and 21was 179.66 ± 8.24, 141.33 ± 8.42 and 73.33 ± 6.44 mm^2, which showed significant reduction ($P < 0.01$) as compared to control group on respective days. Treatment with povidone iodine also showed significant reduction in the wound area (132.33 ± 9.54, 48.50 ± 4.93, 13.16 ± 1.99 and 1.83 ± 0.30 mm^2, $P < 0.001$) on day 8, 12, 16 and 21 as compared to control group. (Figure 1 and 2, Table 1) as well as all study treatements.

Table 1 Effect of treatment of honey, Daruhaldi and Shatdhaut ghrut on wound area in rats.

Days	Control	H	DH	SDG	H + DH	H + SDG	PI
0	420.50 ± 41.09	423.50 ± 23.73	413.50 ± 51.34	415.00 ± 41.13	421.50 ± 16.95	447.50 ± 7.21	429.16 ± 41.79
4	405.83 ± 36.48	389.16 ± 26.50	325.33 ± 23.67	346.50 ± 30.68	319.16 ± 38.33	278.16 ± 7.80	321.83 ± 25.19
8	365.16 ± 32.70	319.50 ± 35.54	231.66 ± 18.79	336.83 ± 32.29	170.33 ± 8.07***	210.33 ± 5.43	132.33 ± 9.54***
12	334.83 ± 28.36	294.33 ± 29.94	147.33 ± 10.74***	320.83 ± 28.36	119.83 ± 16.81***	179.66 ± 8.24**	48.50 ± 4.93***
16	271.50 ± 25.19	164.5 ± 14.85*	80.5 ± 5.86***	245.83 ± 34.00	65.33 ± 7.07***	141.33 ± 8.42**	13.16 ± 1.99***
21	168.33 ± 9.76	113.0 ± 16.52*	41.5 ± 5.03***	154.33 ± 9.76	11.33 ± 2.66***	73.33 ± 6.44**	1.83 ± 0.30***

*Data are expressed as mean ±S.E.M. from six rats and analyze by two Way ANOVA followed by Bonferroni's test. *P < 0.05, **P < 0.01, ***P < 0.001 as compared to vehicle control group rats*

Table 2 Effect of treatment of Honey, Daruhaldi and Shatdhaut ghrut on percent wound contraction in rats.

Days	Control	H	DH	SDG	H + DH	H + SDG	PI
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
4	14.16 ± 1.64	16.00 ± 1.29	42.00 ± 3.07	16.16 ± 1.16	43.50 ± 3.25	34.66 ± 5.47	40.16 ± 3.77
8	15.50 ± 2.81	25.00 ± 2.29	55.16 ± 2.15***	21.66 ± 1.58	55.66 ± 3.93**	40.00 ± 3.87*	70.16 ± 3.47***
12	21.33 ± 2.02	40.83 ± 3.30	56.50 ± 3.43***	31.00 ± 1. 63	79.16 ± 3.30***	51.66 ± 5.49	83.33 ± 2.12***
16	23.33 ± 4.01	46.33 ± 3.37*	61.66 ± 2.14***	38.33 ± 3.15	83.83 ± 4.11***	52.50 ± 4.31**	90.83 ± 1.37***
21	27.00 ± 2.30	50.66 ± 2.10*	84.33 ± 2.18***	42.33 ± 5.84	91.16 ± 2.52***	70.50 ± 1.60**	94.50 ± 1.97***

*Data are expressed as mean ±S.E.M. and analyzed by Two Way ANOVA followed by Bonferroni's test. *P <0.05, **P<0.01, ***P <0.001 as compared to control group animals*

3.3. Effect of treatment of Honey, Daruhaldi and Shatdhaut ghrut on time required for 50% wound closure (CT-50):

As depicted in Table 3, the CT-50 time in the control rats was 38.89 days. Honey and Daruhaldi combination treated rats showed significant decrease in the CT-50 time which was 7.998 days where as Honey and shatdhaut ghrut also showed significant decreased in the CT-50 time i.e. 13.11. In Povidone Iodide treated rats CT-50 time was 7.135 days.

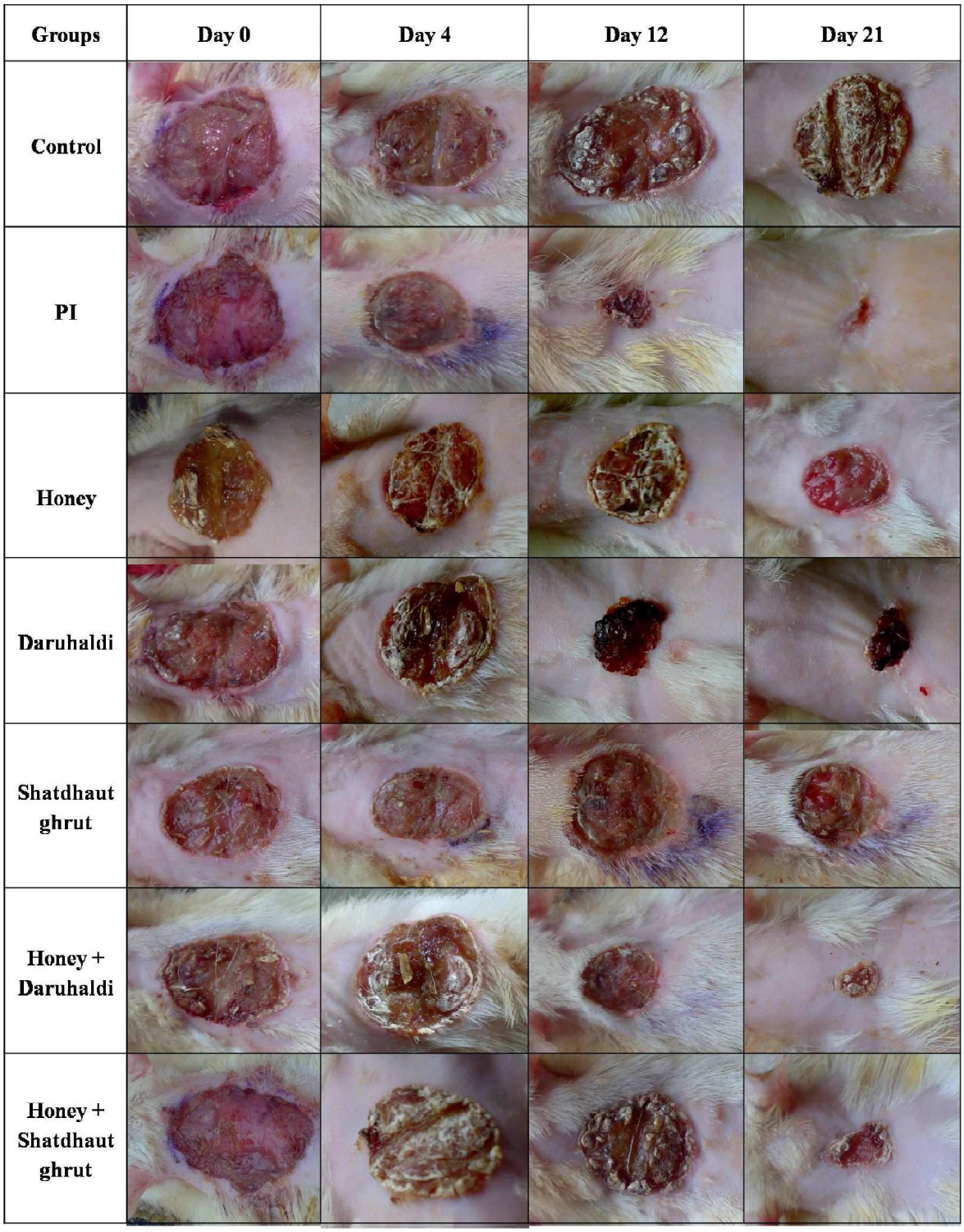

Figure 2 Photographs of rats showing various phases of wound healing

3.2. Effect of treatment of Honey, Daruhaldi and Shatdhaut ghrut on percent wound contraction:

The percent wound contraction in control rats on day 0, 4, 8, 12, 16 and 21 was 0.00 ± 0.00, 14.16 ± 1.64, 15.50 ± 2.81, 21.33 ± 2.02, 23.33 ± 4.01 and 27.00 ± 2.30. Treatment with honey showed significant increase in the percent wound contraction (46.33 ± 3.37 and 50.66 ± 2.10, $P < 0.05$) on day 16 and 21 as compared to control group. Whereas rats treated with daruhaldi showed significant increase in the percent wound contraction (55.16 ± 2.15, 56.50 ± 3.43, 61.66 ± 2.14 and 84.33 ± 2.18, $P < 0.01$, $P < 0.01$, $P < 0.01$ and $P < 0.001$ respectively) on day 8, 12, 16 and 21 as compared to control group. However, shatdhaut ghrut treated rats did not show any significant increase in percent wound contraction as compared to control group. When compared with control group, rats treated with honey and daruhaldi combination showed significant increase in the percent wound contraction (55.66 ± 3.93, 79.16 ± 3.30, 83.83 ± 4.11 and 91.16 ± 2.52, $P < 0.01$ and $P < 0.001$ respectively) on day 8, 12, 16 and 21 as compared to control group. Honey and shatdhaut ghrut combination showed significant increase in percent wound contraction (40.00 ± 3.87, 52.50 ± 4.31 and 70.50 ± 1.60, $P < 0.05$ and $P < 0.01$ respectively) on day 8, 16 and 21 as compared to control group. Treatment with povidone iodide also showed significant increase in the percent wound contraction (70.16 ± 3.47, 83.33 ± 2.12, 90.83 ± 1.37 and 94.50 ± 1.97) on days 8, 12, 16 and 21 as compared to control group and H, DH, SDG & H + SDG. However % wound contraction or % wound closure was comparable in PI & H + DH group on study days 4, 12, 16 & 21. (Figure 3 and Table 2)

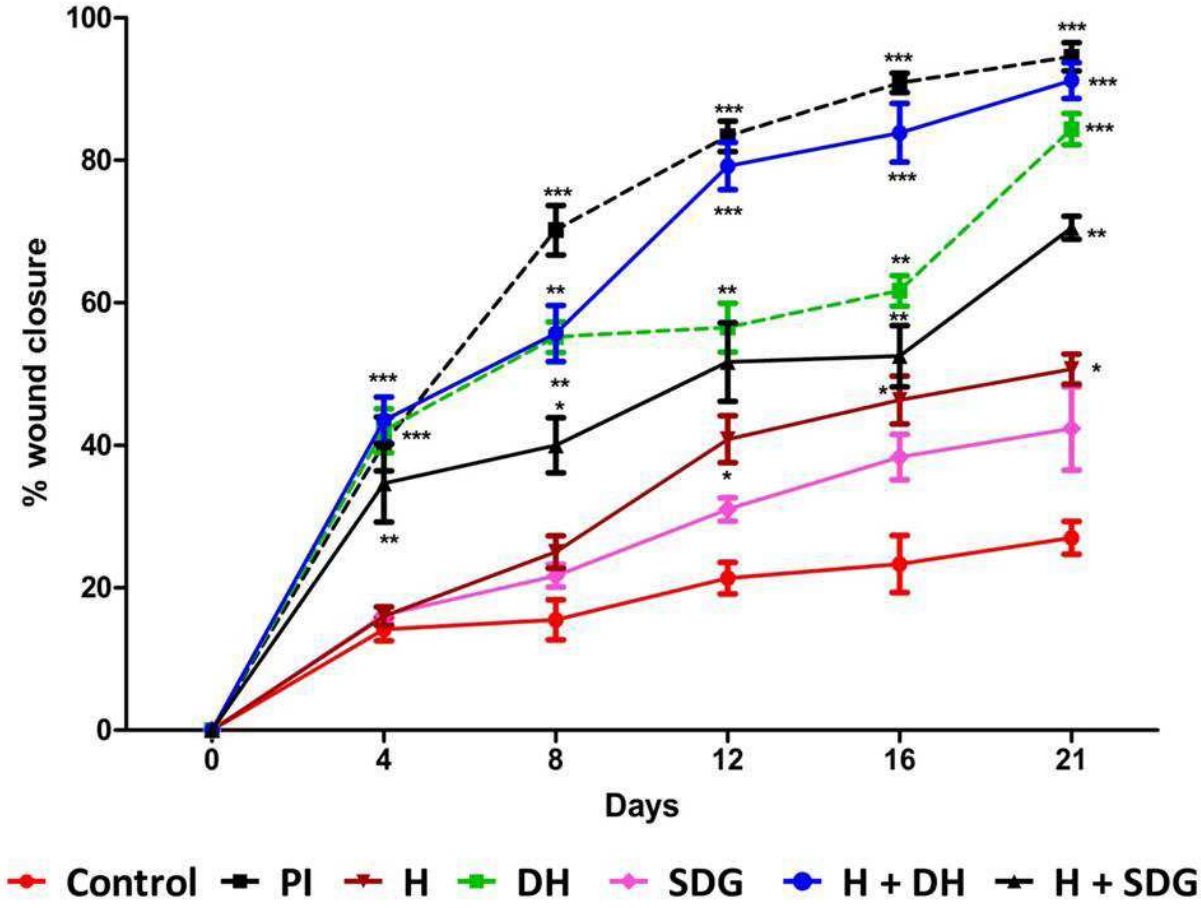

Figure 3 Effect of treatment of Honey, Daruhaldi and Shatdhaut ghrut on percent wound contraction in rats.

*Data are expressed as mean ±S.E.M. from six rats and analyze by two Way ANOVA followed by Bonferroni's test. *P < 0.05, **P < 0.01, ***P < 0.001 as compared to vehicle control group rats*

Table 3 Effect of treatment of Honey, Daruhaldi and Shatdhaut ghrut on time required for 50% wound closure (CT-50)

Treatment	Control	Povidone Iodine	SDG	H	DH	H + DH	H + SDG
CT-50(in Days)	38.89	7.135	22.90	18.36	10.18	7.998	13.11

The graph of % wound closure versus time in days was plotted in software (Graphpad Prism v 5) and linear regression analysis was performed. The value of X at Y=50% was taken as CT50 (half-closure time, Time taken to close the wound by 50%) (n = 6)

DISCUSSION

The present study was planned to evaluate therapeutic potential of effect of Honey, Daruhaldi & Shatdhdhaut ghrut alone and in combination on experimental excision would healing activity in rats. 3 parameters were used wound area, percent wound contraction and CT 50.

Wound healing is a natural process which comprises of the various phases including collagenation, wound contraction and epithelization which are interlinked with each other. Interference with any one of these phases by the drug leads to delay in wound healing or may promote wound healing [6].

Control group did not receive any treatment, since wound healing is a natural process. PI commonly used clinically for wound dressing, was used as standard control. PI showed good wound healing activity on all parameters. In this study H + DH combination showed better wound healing activity as compared to other test drugs. Effect of this combination on wound area was less as compared to PI. However effects of H + DH & PI were comparable as seen in % wound contraction. This can be explained by the fact on day '0' wound area may differ but this is nullified in % wound contraction. Although wound area on day '0' with all study treatments did not show significant difference. In this excision wound model SDG alone or in combination with honey did not show good wound healing activity as compared to PI. This study was not planned to elucidate the mechanism of action of the test drugs but effect of honey on wound healing and its probable mechanism of action has been reported in literature by Gabbiani *et al*. [25].

It has been well documented that elevated levels of reactive oxygen species and lipid peroxidation play an important role in the skin lesions and in modulation of fibroblast proliferation [26-28]. Infliction of wound results in decreased antioxidant efficiency of the tissue which makes it more vulnerable to free radical attack [29, 30]. The drugs with antioxidant profile play a vital role in the wound healing process through the down regulation of elevated levels of free radicals [31, 32].

Honey has long been used for treating wounds and other skin conditions [16, 33]. It has been documented that honey is active against antibiotic-resistant pathogens and acts as an antimicrobial agent [19, 34, 35]. Gheldof *et al*. as well as Gheldof and Engeseth have shown that many of the compounds in honey have antioxidants property [36, 37]. In this study antibacterial action of honey may not be significant since the wounds were clean & not infected. Treatment with honey also increases the thickness of the granulation tissue and the area of re-epithelization [38].

Berberis aristata (Dauhaldi) is a potential antioxidant agent [39]. It's an antibacterial agent and acts through DNA damage during cellular proliferation of bacteria [40]. This antioxidant and antibacterial property of Daruhaldi might play important role in the wound healing process. It has been communicated that flavonoid derived from plant source has anti-lipidperoxidation potential which helps in improving vascularity and decreasing the cell necrosis [41-45]. The intense yellow color of coptis chinensis root is most likely due to the high content of berberine, which is bitter in taste. Berberine present in the *Berberis aristata* might promote the wound healing process [46]. Therefore wound healing activity as observed with H & DH may be because of antioxidant property of honey as well as Daruhaldi. The synergistic acitivity of these two drugs may be explained by action of Honey on the thickness of granulation tissue & area of reepithelisation as described by Postmes *et al*. [47]

In clinical trial CT-50 is utilized for Phase II trials in determination of chronic ulcers to test efficacy [48]. Treatment with Honey and Daruhaldi as well as Honey.

The improved wound healing potential and synergistic effect of Honey & Daruhaldi can be attributed to additive, antioxidant potential of Honey and flavonoid moities in Daruhaldi combination showed better wound healing potential than Honey & Daruhaldi alone.

REFERENCES

[1] Enoch S, John Leaper D, *Surgery*, **2005**,23, 37-42.
[2] Ayello EA, *Adv Skin Wound Care* **2005**,18,98-109.
[3] Senthil Kumar M, Sripriya R, Vijaya Raghavan H, Sehgal P, *J Surg Res* **2006**,131, 283-9.
[4] Mazumdar BC, Mukhopadhyay PM, Daya publishing house, Delhi, **2006**, pp. 1.
[5] Sumitra M, Manikandana P, Suguna L, *Int J Biochem Cell Biol* **2005**,37,566-73.
[6] Patil MVK, Kandhare AD, Bhise SD, *Asian Pac J Trop Biomed* **2012**:S646-S655.
[7] Diegelmann RF, Evans MC, *Front Biosci **2004***:9,283-9.
[8] Fulzele SV, Satturwar PM, Joshi SB, Dorle AK, *Indian Drugs* **2002**:39,606-9.
[9] Hunt TK, *Int Ann Surg* **1969**:170,633-41.
[10] Patil MK, Kandhare AD, Bhise SD, *Chron Young Sci* **2011**:2,207-13.
[11] Kandhare AD, Raygude KS, Ghosh P, Gosavi TP, Bodhankar SL, *Int J Pharm Biol Arc* **2011**:2(4),1024-1032.
[12] Duke JA, Bogenschutz-Godwain MJ, Ducellier J, Duke PK, CRC press, London. **2002**.
[13] Lynch SE, Nixon JC, Colvin RB, Antoniades HN, *Proc Natl Acad Sci USA* **1987**: 84(21),7696–7700.
[14] Pierce GF, Mustoe TA, Altrock BW, Deuel TF, Thomason A, *J Cell Biochem* **1991**:45(4),319-26.
[15] Pandey M, Worlikar PS, Ghosh A, Bondekar AA, Chetan S, *Int J Health Allied Sci* **2012**: 1(2), 59-63.
[16] Efem SC, *Br J Surg* **1988**:75,679-81.
[17] Subrahmanyam M, *Br J Surg* **1991**:78,497-8.
[18] Ali AT, *Scand J Gastroenterol* **1991**:26,281-8.
[19] Allen KL, Molan PC, Reid GM, *J Pharm Pharmacol* **1991**:43,817-22.
[20] Oztasan N, Altinkaynak K, Akcay F, Gocer F, Dane S, *Turk J Vet Anim Sci* **2005**:29,1093-6.
[21] Kirtikar KR, Basu BD, Indian Medicinal Plants. 2nd ed. Vol. I, Dehradun, India: International Book Distributors: **1984**.
[22] Nadkarni AK. Indian Materia Medica-revised an enlarged. 3rd ed. Vol. II. Bombay: Popular Book Depot: **1976**.
[23] Gupta SK, Agarwal R, Srivastava S, Agarwal P, Agrawal SS, Saxena V, Galpalli X, *Invest Ophthalmol Vis Sci* **2008**:49(9),4036-40.
[24] Deshpande S, Deshpande A, Tupkari S, Agnihotri A, *Indian J Trad Knowledge* **2009**:8(3):387-91.
[25] Gabbiani G, Harschel BJ, Ryan GB, *J Exp Med* **1976**: 135,719.
[26] Murrell GAC, Francis MJO, Bromley L, *Biochem J* **1990**:265,659-65.
[27] Kandhare AD, Raygude KS, Ghosh P, Ghule AE, Bodhankar SL, *Neurosci Lett* **2012**:511,18-22.
[28] Kandhare AD, Raygude KS, Ghosh P, Ghule AE, Gosavi TP, Badole SL, Bodhankar SL, *Asian Pac J Trop Biomed* **2012**:5,337-344.
[29] Shukla A, Rasik AM, Patnaik GK, *Free Radic Res* **1997**: 26,93-101.
[30] Maiere CM, Chan PH, *Neuroscientist* **2002**:8,323-4.
[31] Kandhare AD, Raygude KS, Ghosh P, Ghule AE, Bodhankar SL, *Fitoterapia* **2012**:83,650-9.
[32] Kandhare AD, V. Shiva K, Mohammad A, Rajmane AR, Ghosh P, Bodhankar SL, *Orient Pharm Exp Med* **2012**:12(4), 287-299.
[33] Phuapradit W, Saropala N, *Aust NZ J Obstet Gynaecol* **1992**:32,381-84.
[34] Molan PC. The antibacterial activity of honey, *Bee World* **1992**:73,5-28.
[35] Cooper RA, Molan PC, Harding KG, *J Roy Soc Med* **1999**:92,283-5.
[36] Gheldof N, Engeseth NJ, *J Agric Food Chem* **2002**:50,3050-5.
[37] Gheldof N, Wang XH, Engeseth NJ, *J Agric Food Chem* **2002**:50,5870-7.
[38] Bangroo AK, Kharti R, Chauhan S, *J Indian Assoc Pediatr Surg* **2005**:10,172-5.
[39] Singh J, Kakkar P, *J Ethnopharmacol* **2009**:123(1),22-6.
[40] Mundada AS, Mahajan MS, Gangurde HH, Borkar VS, Gulecha VS, Khandare RA, *Pharmacologyonline* **2009**:2,1185-91.
[41] Getie M, Gebre-Mariam T, Rietz R, Neubert RHH, *Pharmazie*, **2002**:57(5),320-2.
[42] Shetty S, Udupa S, Udupa L, *Evid Based Complement Alternat Med* **2008**:5(1),95-101.
[43] Kandhare AD, Raygude KS, Ghosh P, Bodhankar SL, *Int J Green Pharm* **2011**:5,236-43.
[44] Kandhare AD, Raygude KS, Ghosh P, Ghule AE, Zambare GN, Bodhankar SL, *Apollo Medicine* **2013**:10,87-97.

[45] Patil MVK, Kandhare AD, Bhise SD, *Biomed Aging Pathol* **2012**:2,6-15.
[46] Mazumder PM, Das S, Das S, Kumardas M, *Int Bull Drug Res* **2012**:1(1), 47-53.
[47] Postmes TJ, Bosch MMC, Dutrieux R, van Baare J, Hoekstra MJ, **1997**. pp. 27-37.
[48] Engelmayer J, Blezinger P, Varadhachary A, *J Surg Res* **2008**:149(2),278-86.