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Wound Healing activity of *Durva ghrita*

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***Durva ghrita* is herbal formulation containing *Cynodon dactylon* and Cow's *ghee* as its main constituents. In the present study *Durva ghrita* was evaluated for its wound healing property by incision and excision wound models in male Wistar rats. Treatment with *Durva ghrita* alone promoted wound contraction and reduced the time for closure showing healing potential comparable to framycetin sulphate cream 1% w/w. Histopathological studies shows proliferation of epithelial tissue, angiogenesis and fibrosis due to treatment with *Durva ghrita*. The present study demonstrates wound healing potential of the test formulation.**

Wounds are visible results of individual cell death or damage, and can be classified by site, size, depth and causation–surgery, accident or circulatory failure[1]. Wound healing is a process which is fundamentally a connective tissue response. Initial stage of this process involves an acute inflammatory phase followed by the synthesis of collagen and other extracellular macromolecules which are later remodeled to form a scar[2,3]. The present study was initiated to evaluate the wound healing activity of *Durva ghrita* based on its use for wound healing in traditional practices[4]. *Durva ghrita* is a herbal formulation belonging to *panchgavya* class of Ayurvedic preparations. *Panchgavya* refers to the five important products of bovine origin *viz.* milk, curd, *ghee*, urine and dung. Literature thus far has documented only a few pharmacological actions and clinical uses of *Panchgavya* formulations[5,6]. However, no systematic study has been attempted to confirm the traditional practice of using *Durva ghrita* for wound healing. The present study has been taken up to provide experimental evidence for the wound healing activity possessed by *Durva Ghrita*.

MATERIALS AND METHODS

Durva ghrita used in the present investigation is composed of cow's *ghee* (2.5%) and *Cynodon dactylon* (10%). *Durva ghrita* was obtained as a gift sample from Go-Vigyan Anusandhan Kendra, Nagpur.

Experimental protocol:

Male Wistar rats weighting 150-200 g were used. They were individually housed and maintained under standard environmental condition of temperature (23± 1°), 12 h light/dark cycle and fed on normal pellet diet (Gold Mohur brand, Lipton India Limited) and water *ad libitum*. Animals were acclamatized to laboratory conditions before experiments were carried out. Rats were divided into three groups of 6 animals each as follows: group 1, untreated control; group 2, treated topically with *Durva ghrita* and group 3, treated topically with framycetin sulphate cream (FSC) 1% w/w[4]. Animals were housed individually in clean polypropylene cages. The experimental protocols were approved by the Institute Animal Ethics Committee. Except the drug under study no topical or systematic therapy was given to animals subjected with any of the wounds. Animals showing infection/deterioration of wounds were excluded from the study and replaced with new animals.

Excision wounds:

Excision wounds were inflicted in rats as described by Morton and Malone[7] under light ether anesthesia. The skin of the impressed area was excised to the full thickness to obtain a wound area of about 300 mm². The parameters

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TABLE 1: EFFECT OF TOPICAL APPLICATION OF *DURVA GHRITA* ON EXCISION WOUNDS.

Post wounding days	Wounding area (mm^2)		
	Group 1	Group 2	Group 3
0	379±9.26 (0)	291±2.71* (0)	345±6.63* (0)
3	280±8.21 (25.9)	262±18.4* (10.0)	300±6.63* (12.9)
6	246±8.21 (35.1)	240±6.0* (17.6)	243±5.41* (29.5)
9	210±7.79 (44.5)	199±3.46* (31.8)	200±2.93* (53.1)
12	193±3.05 (53.4)	151±1.92* (49.0)	162±5.06 (53.1)
15	177±3.05 (53.4)	112±8.35 (62.7)	121±4.47 (65.0)
18	151±1.54 (60.1))	80.9±7.3 (72.2)	80.2±0.44 (76.8)
21	122±0.14 (67.9)	50.3±2.25 (82.8)	29.8±0.08 (91.4)
24	101±0.15 (73.3)	20.3±6.39 (93.0)	0.0 (100)
27	60.7±0.08 (84.0)	0.0 (100)	0.0 (100)

Values of mean±SD of animals in each group. Number in paranthesis indicates percentage of wound contraction. All values are significant at $P<0.05$ as compared to Group I and * indicate not significant.

studied were wound closure and contraction time[8]. The percentage wound closure was recorded on 0, 3, 6, 9, 12 and 16 day till wounds were completely healed[9]. The scar shape and area were traced and measured planimetrically. The wound size of 300 mm^2 was taken as 100% and scar area was expressed as a percent of the original wound size[10].

Incision wound:

Two 5 cm long paravertebral incisions were made through the entire thickness of skin at a distance about 1.5 cm from midline on each side of depilated back of the rat[11]. The wounds are closed with interrupted sutures which were removed on day 8 and on day 10 the tensile strength was measured[12].

Histopathological Studies:

Sections from regenerated tissue (10 d) were studied under light microscope for keratinization, epithelization, fibrosis, inflammation and neovascularization. The result studied were numbered from 1 to 5 with 5 standing for maximum similarity and 1 standing for least similarity from normal tissue around the wound area in test and untreated wounds.

Statistics:

Results have been expressed as mean±S.D. Statistical significance of the difference of drug treated group in compare to control group was evaluated using one way analysis of variance, (ANOVA)[13] followed by Tukey Kramer Multiple Comparisons test ($p<0.05$).

RESULTS AND DISCUSSION

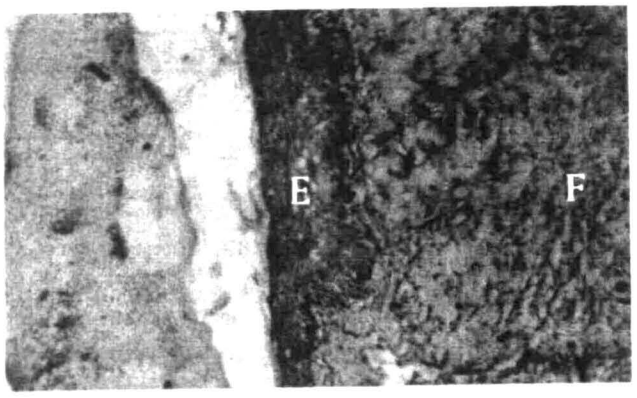
In the above study the results for the progress of wound healing in excision wound are recorded in Table 1. After treatment with *Durva ghrita* the results are matched with that of FSC 1% w/w, with both showing better healing compared to untreated control ($P<0.05$). The wound contraction ceased around 21-24 d but the treatment was continued upto d 30 to monitor fall of eschar, leaving no raw wound behind. Observations suggest that the wound healing occurred around d 21-24 followed by fall of eschar.

TABLE 2: EFFECT OF TOPICAL APPLICATION OF *DRUVA GHRITA* ON INCISION WOUNDS.

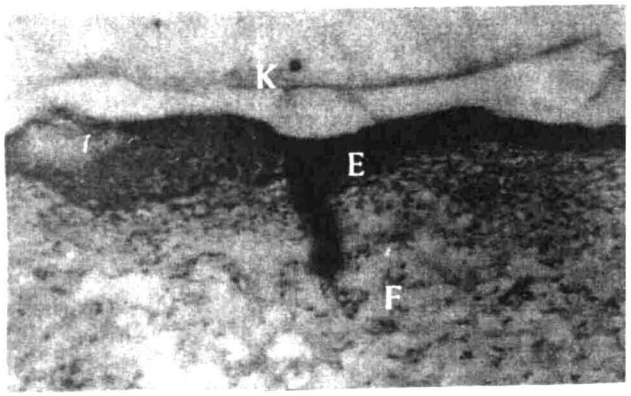
Groups	Treatment	Tensile strength (g)
1	Untreated	281±5.82
2	Durva ghrita	337±5.12*
3	FSC 1% w/w	398±6.22*

Values are mean ±SD of 6 animals in each groups. *$P<0.05$ vs Group 1.

In the incision wound model, results of tensile strength measurement are shown in Table 2. In incision wound studies, there was a significant increase in tensile strength



a)



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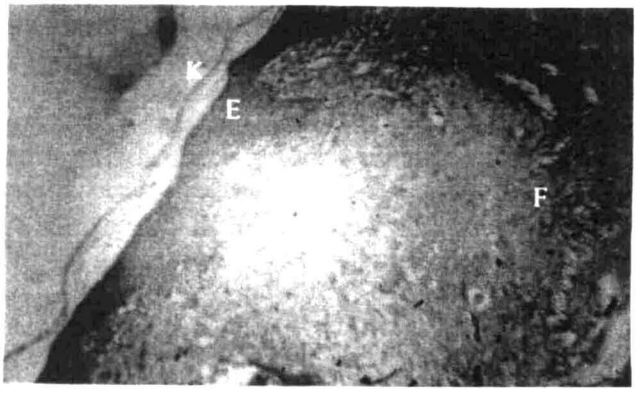

c)

Fig. 1: Histopathology of 10 d old regenerated tissues. Photomicrographs of histology of regenerated tissue collected from (a) untreated control, (b) *Durva ghrita*-treated and (c) FSC (1% w/w)-treated after 10 d. Magnification is 100X. K denotes keratinization, E indicates epithelization; and F stands for fibrous tissue.

TABLE 3: HISTOPATHOLOGICAL EXAMINATION OF WOUNDS TREATED WITH *DRUVA GHRITA* AT END OF 10D.

Parameters	Treatment		
	Group 1	Group 2	Group 3
Keratinization	0.3±0.12	1.1±0.11	4.3±0.11
Epithelization	1.6±0.21	4.3±0.19	4.1±0.22
Fibrosis	2.1±0.23	4.4±0.25	4.2±0.38
Collagenation	2.6±0.41	3.9±0.18	4.3±0.29
Neovascularization	0.6±0.29	3.3±0.19	4.4±0.37

Values are mean±SD from 6 readings each. Values 5 refers to maximum similarity and 0 refers for least similarity of wound from the normal tissue. All values are significant at $P<0.05$ as compared to Group 1.

of 10 d old wound due to treatment with *Durva ghrita* and it is mostly same with FSC, both showing significant increase over untreated controls. The mean±SD of tensile strength in control animals was 281±5.82 while in *Durva ghrita* it is 337±5.12 and in FSC 398±6.32. A significant reduction in the period of epithelization was observed when compared with group 1. Significant increase in tensile strength of incision wound suggest that *Durva ghrita* promotes collagen formation, thereby promoting faster healing than untreated wounds.

Histopathological observations of the regenerated tissue shows incomplete healing with less keratinization, epithelization, fibrosis and collagen formation in untreated rats. With *Durva ghrita,* recorded healing parameters are shown in Table 3. The results for *Durva ghrita* indicate that keratinization is comparable to FSC 1% w/w. Epithelization, fibrosis, collagenation and neovascularisation are also comparable with FSC 1% w/w treatment. This can easily be seen with fig. 1(a) for untreated control, fig. 1(b) for *Durva ghrita* treatment and fig. 1(c) for FSC 1% w/w treatment. From this it may be concluded that *Durva ghrita* promotes epithelization, fibrosis, collagenation and neovascularisation comparable with treated control.

The process of wound healing occurs in four phases (i) coagulation, which prevent blood loss, (ii) inflammation and debridement of wound, (iii) repair, including cellular proliferation and (iv) tissue remodeling and collagen disposition[14]. The primary mechanism by which the test formulation in this study seems to promote the wound

healing is by collagen formation and tissue remodeling. The results of tensile strength support this. From the above study it may be inferred that Durva *ghrita* promotes wound healing in excision as well as incision models rationalizing its traditional claim.

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