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Article

Effect of *Ficus hispida* L. on normal and dexamethasone suppressed wound healing

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Ethanolic extract of roots of *Ficus hispida* was investigated in normal and dexamethasone depressed healing conditions, using incision, excision and dead space wound models in albino rats. The root extract of *Ficus hispida* has shown the maximum breaking strength compared to control group. The rate of epithelialization and wound contraction in excision model was better as compared to control groups. There was significant increase in granulation tissue weight and hydroxyproline content in dead space model compared to control group. The antihealing effect of dexamethasone was also reverted by the administration of ethanolic extract of *Ficus hispida* in all the wound models .The results indicated that the root extract of *Ficus hispida* has a significant wound healing activity and also promotes healing in dexamethasone depressed healing conditions.

Uniterms: *Ficus hispida*/pharmacognosy. *Ficus hispida*/ethanolic extract/wound healing activity. Wound healing/experimental study.

O extrato etanólico de raízes de *Ficus hispida* foi ensaiado em ratos albinos normais e em condições de cicatrização deprimida por dexametasona, utilizando modelos de ferida por incisão, excisão e de espaço morto. O extrato da raiz de *Ficus hispida* mostrou a força máxima de tensão comparativamente ao grupo controle. A velocidade de epitelização e de contração da ferida no modelo de excisão foi melhor do que a dos grupos controles. Houve aumento significativo no peso do tecido de granulação e no conteúdo de hidroxiprolina no modelo de espaço morto comparativamente ao grupo controle. O efeito anticicatrizante da dexametasona foi, também, revertido pela administração do extrato etanólico de *Ficus hispida* em todos os modelos de feridas. Os resultados indicaram que o extrato de *Ficus hispida* tem atividade cicatrizante em feridas e, também, promove a cicatrização em condições de depressão de cicatrização pela dexametasona.

Unitermos: *Ficus hispida*/Farmacognosia. *Ficus hispida*/extrato etanólico/atividade cicatrizante. Cicatrização/estudo experimental.

INTRODUCTION

A wound may be defined as a break in the epithelial integrity of the skin or may also be defined as a loss or breaking of cellular and anatomic or functional continuity of living tissue (Ramzi *et al.*, 1994). Studies on wound healing aim to detect various means and factors influencing the healing process, so they could be either used or avoided in clinical practice to favorably alter the healing process (Stuart *et al.*, 2004). Although many indigenous tribes around the world have long suspected that *Ficus hispida* might have medicinal wound healing properties, it has not really got the attention of orthodox medical practitioners as a potential source of a healing agent which may prove to be useful in the treatment of wounds (Kurian *et al.*, 1994).

Some plants possessing pro-healing activity have been scientifically analyzed. The wound healing potential of *Tridax procumbens* (Udupa *et al.*, 1995), *Trigonella foenumgraecum* (Taranalli, Kuppast, 1996), *Leucas lavandulaefolia* (Saha *et al.*, 1997), *Aloe vera* (Chitra *et al.*, 1998), *Ageratum conyzoides* (Chah *et al.*, 2006), *Dendrophthoe falcate* (Pattanayak *et al.*, 2008) and *Heliotropium indicum*, *Plumbago zeylanicum, Aca-*

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lypha indica (Reddy *et al.*, 2002) have shown promising healing activity.

Research on wound healing drugs is a developing area in modern biomedical sciences. Scientists who are trying to develop newer drugs from natural resources are looking toward the Ayurveda, the Indian traditional system of medicine. Several drugs of plant, mineral, and animal origin are described in the Ayurveda for their wound healing properties under the term Vranaropaka. Most of these drugs are derived from plant origin. Some of these plants have been screened scientifically for the evaluation of their wound healing activity in different pharmacological models and patients, but the potential of most of them remains unexplored. The present day requires a new biologically active drug that exhibits wound-healing activity, so as to increase the wide spectrum of medicinal usages.

Ficus hispida L. belongs to the Moraceae family. It is a moderate sized tree, that grows up to 3.0 m, with spreading branches and many aerial roots. It is widely distributed throughout India, Srilanka, Myanmar, Southern region of the Republic of China, New Guinea, Australia and Andaman Islands in damp localities. It also grows in secondary forests, open lands and riverbanks up to 1200 m in altitude (Ripu *et al.*, 2006). *Ficus hispida* is used by the maaiba indigenous medicine - man of Manipur, India as an indigenous traditional medicine (Manandhar, 1995). The extracts of all parts of the plant have been reported to be bitter, cooling, astringent and antidysenteric and to have activity against psoriasis, anemia, piles, jaundice, and hemorrhage. (Nadkarni, 1976; Rastogi, Mehrotra, 1993). The fruit acts as a coolant and tonic. The juice obtained from the fig is taken along with jaggery as a mild purgative. A mixture of honey and its juice is a good antihaemorrhagic (Sergio *et al.*, 2002)and the root and leaves are of particular interest, from a medicinal point of view, as an antidiarrhoeal (Subhash, Mandal, 2002).

Until today, there are no effective drugs available which can successfully reverse the dexamethasone depressed healing. Dexamethasone is a potent anti-inflammatory glucocorticoid used in transplantation and allograft rejection (Tripathi, 1999). Glucocorticoids are known to suppress wound healing (Ehrlich, 1969).

Previous studies showed that *F. hispida* leaves contain oleanolic acid, bergapten, β-amyrin, β-sitosterol, hispidin and phenanthroindolizidine alkaloids (Huong and Trang, 2006). Roots of Ficus hispida contain mainly alkaloids like β-amyrin, β-sitosterol, and hispidin (Ayurvedic pharmacopoeia of India).

Therefore, the current study was undertaken to evaluate the wound healing effect alone and in the presence of dexamethasone-induced suppression in rats by the oral administration of ethanolic extract of *Ficus hispida* root.

MATERIALS AND METHODS

Materials

The root of *Ficus hispida* was collected from adjoining areas of Modinagar (Ghaziabad), in July, 2009, and was authenticated by Associate Professor Dr. M. K. Saxena, Department of Botany, M.P.G.College campus, Modinagar, C.C.S University, Meerut (U.P), through macroscopic and microscopic evaluation and the exsiccate was deposited in the Department of Pharmacognosy, Dr.K.N.Modi Institute of Pharmaceutical Education & Research centre, Modinagar, India, for future reference (Specimen Number: *F.H* 01/07/2009).

Preparation of the root extract

Roots of the plant were dried in shade for one week. The dried root barks were powdered (3 kg) defatted with petroleum ether (60–80 ^{0}C), soaked in ethanol (95%) and macerated for 4 days. After 4 days, the ethanolic layer was decanted off. The process was repeated for four times. The solvent from the total extract was distilled off by vacuum distillation.

The preliminary phytochemical analysis

The preliminary phytochemical studies were performed for testing different chemical groups present in ethanolic extract (Trease, Evans, 1987). The freshly prepared root extract of *Ficus hispida* (FH) was qualitatively tested for the presence of chemical constituents. Phytochemical screening of the extract was performed for alkaloids, flavonoids, phenolic compounds and tannins, carbohydrate, proteins and amino acids. Saponin was also tested. These were identified by characteristic color changes using standard procedures.

Animals

Wistar albino rats of either sex weighing between 180 and 200 g were obtained from Nitin Scientic Lab, New Delhi. The study was approved by the Institutional Ethics Committee for animal experimentation Dr.KNMIPER, Modinagar (Dr.KNMIPER/ IEAC/ CPCSEA/ 2009/ 19), and all the procedures on animals were carried out according to CPCSEA guidelines, India. These animals were used for the wound healing activity studies. The animals were

stabilized for 1 week. They were maintained in standard conditions at room temperature, 60±5% relative humidity and 12 h light-dark cycle. They had been given standard pellet diet and water *ad libitum* throughout the course of the study. The ethanolic extract of *Ficus hispida* was administered orally (p.o.); whereas dexamethasone (Dexona Vials) was administered intra peritoneal (i.p).

Excision wound model

Under light ether anesthesia an impression of 500 sq mm was made on the shaved back of the rat as described (Morton and Malone, 1997). The skin of the impressed area was excised carefully. Animals were kept in separate cages. The day on which wound was made was considered as day '0' (Zero) (Table - I). Wound area was traced and measured planimetrically with the help of sq mm graph paper. The period of epithelization was calculated as the number of days required for falling of the eschar without any residual raw wound. The parameters which were observed were the period of epithelialization (Reepithelialization).

Incision wound model

The rats were anesthetized by administering ketamine (0.5 mL/kg b. w. i.p.). Incision wounds of about 6 cm in length and 2mm in depth were made with sterile scalpel on the shaved back of the rats 30 min after the administration of the ketamine injection. The parted skin was kept together and stitched with black silk at 0.5 cm intervals. A surgical thread (no. 000) and a curved needle (no. 9) were used for stitching. The continuous thread on both wound edges was tightened for good closure of the wounds. The wounds of animals in the different groups were treated with the drug by oral administration as described above, for the period of 10 days. The wounding day was considered as day 0. When wounds were cured thoroughly, the sutures were removed on the 8th post-wounding day and the tensile strength of the skin, which is the weight in grams required to break open the wound/skin, was measured by tensiometer on the 10th day reported (Nath *et al.*, 2006).

Tensile strength was calculated using the following formula (Diwan *et al.*, 2008):

$$\textbf{Tensile strength} = \frac{\text{Breaking strength (g)}}{\text{Cross-sectional area of skin (mm}^2\text{)}}$$

Dead space wound model

The dead space wounds were created by making a small transverse incision in the lumber region on either side of the vertebral column in each animal. Two polypropylene tubes (2.5 cm × 0.5 cm) were inserted subcutaneously, one on either side of the vertebral column, and pushed cephalad for 3 cm - 4 cm for the final implantation to harvest the granulation tissue. The animals were treated with the extracts from 0 day to 9th post-wounding day considering wounding day as zero. Granulation tissue formed on the implanted tubes was carefully dissected out on the 10th post-wounding day and the tensile strength was measured by continuous constant water flow technique (Lee, 1968). Mean value gives the breaking strength for a given group. The tissue was dried in oven at 60 °C for 24 hours and the dry weight was noted. The acid hydrolysate of the dry tissue was used for the estimation of the hydroxyproline content in the tissue (Neuman, 1950).

Statistical analysis

The mean value ± SEM was calculated for each parameter. Results were statistically analyzed by one-way-analysis of-variance (ANOVA) followed by posthoc scheffe's test. $P < 0.05$ was considered as significant.

RESULTS

In incision wound model, the drug-treated group (Group 2) showed significant breaking strength compared to control group (Table I) and the dexamethasone-treated (Group 3), showed significant decrease in breaking strength. But, comparatively, the ethanolic root extract showed a promising reversal of dexamethasone depressed healing in rats by increasing the breaking strength in incision wound model (Group 4).

In dead space wound model, the ethanolic extract significantly increased the hydroxyproline content, which is a marker of collagen content and thus, of the healing process (Table I)**.** In this model, the dry and wet granulation tissue weight and granulation tissue breaking strength were increased compared with control group and dexamethasone group.

In excision wound model, the ethanolic root extract of *Ficus hispida* showed a promising result by decreasing the period of epithelialization (Table I) and increasing the percentage of wound contraction (Table II) compared to control group. The dexamethasone-treated group showed increase in epithelialization period and the percentage of wound contraction was decreased. But, the ethanolic extract has significantly reverted the period of epithelialization by increasing it, and the percentage of wound contraction was also increased (Table II).

TABLE I - The effect of Ethanolic extract of *Ficus hispida* in absence and in presence of Dexamethasone treated rats in incision, excision and Dead space wound models [Values are Mean ± SD for 6 rats]

Groups	Incision wound breaking strength	Excision wound period of epithelialization (days)	(Dead space model) Wet Tissue Weight (mg/100g rat)	(Dead space model) Dry Tissue Weight (mg/100g rat)	(Dead space model) Breaking Strength (g)	(Dead space model) Hydroxyproline Content (mg/g tissue)
Control	270.7± 24.38	23 ± 1.57	245 ± 21.45	29 ± 2.0	288 ± 14.78	12.67 ± 3.21
Ficus hispida Ethanolic Treated (p.o.)	442.2 ± 33.66^{a}	16 ± 1.21^{a}	345 ± 15.50^{a}	47 ± 1.5^{a}	399 ± 17.8^{a}	49.34 ± 7.12^{a}
Dexamethasone Treated (i.p.)	205 ± 23.67^{c}	35 ± 5.23^{ax}	180 ± 11.25^{bx}	20 ± 3.4^{x}	180 ± 9.6^{ax}	9.67 ± 2.67^{x}
Ethanolic Extract + Dexamethasone Treated	340 ± 15.67^{c}	25 ± 1.35^{xp}	260 ± 17.62^{xp}	35 ± 5.0^{yr}	268 ± 11.3^{xp}	19.54 ± 6.23^{x}

[P Values: ^{a} < 0.001, ^{b} <0.01, ^{c} < 0.05 Vs Control, ^{x} < 0.001, ^{y} < 0.01, Vs *F. hispidus,* ^{p} < 0.001, ^{r} < 0.05, Vs Dexamethasone].

TABLE II - The effect of Ethanolic extract of *Ficus hispida* in absence and in presence of Dexamethasone-treated rats in excision wound model for wound contraction [Values are Mean ± SD for 6 rats]

Groups	Percentage of wound contraction in days				
	4th Day	8th Day	12th Day	16th Day	20th Day
Control	10 ± 2.36	45 ± 2.23	69 ± 1.78	85 ± 4.1	96 ± 4.0
Ficus hispida Ethanolic Treated (p.o.)	30.45 ± 4.8^{a}	65.67 ± 4.8^{cx}	88.78 ± 3.23	99.67 ± 1.2^{bx}	99.95±0.7^{ax}
Dexamethasone Treated (i.p.)	5.7 ± 3.2^{ax}	15.43 ± 5.8^{ax}	38.34 ± 5.5^{ax}	59.67 ± 5.67^{ax}	71.34 ± 4.67^{bx}
Ethanolic Extract + Dexamethasone treated	8.89 ± 2.1^{ax}	25.78 ± 2.34^{ax}	49.78 ± 3.46^{ax}	79.78 ± 5.5^{cy}	88.45 ± 3.43^{r}

[P Values: ^{a} < 0.001, ^{b} <0.01, ^{c} < 0.05 Vs Control, ^{x} < 0.001, ^{y} < 0.01, Vs *F. hispidus,* ^{p} < 0.001, ^{r} < 0.05, Vs Dexamethasone].

DISCUSSION

The wound healing process consists of different phases such as granulation, collagenization, collagen maturation and scar maturation, which are concurrent but independent to each other. Hence, in this study three different models were used to assess the effect of ethanolic *Ficus hispida* root extract on various phases.

Oral administration of *Ficus hispida* at the wound site produced significant wound healing activity. Its pro-healing activity was conspicuous as all the observed healing parameters were significantly affected. As expected, after 7 days of treatment with plant extract, the wounds exhibited marked dryness of wound edges with regeneration of healing tissue and the wound area was also considerably reduced compared to controls indicating the healing potential of *Ficus hispida.* (Udupa *et al.*, 1995).

Further raised levels of hydroxyproline by about 50% in regenerated tissue suggest enhanced collagen synthesis, an important constituent of extracellular matrix. Collagen not only confers strength and integrity to the tissue matrix, but also plays an important role in haemostasis and in epithelization at the later phase of wound healing (Clark, 1996). Hence, enhanced collagen synthesis by *Ficus hispida* may significantly contribute to healing and also provide necessary strength to repaired tissue.

The extract also appears to stimulate significant reduction in wound size, which might be due to enhanced epithelization. Therefore it appears that the *Ficus hispida* extract possesses significant pro-healing activity by affecting healing at the various phases of tissue repair.

The preliminary phytochemical analysis of the *Ficus hispida* root extract showed the presence of tannins, alkaloids and saponins, which may be responsible for the wound pro-healing activity.

In excision wound model, dexamethasone-treated

animals showed significant increase in the epithelization period and decreased percentage of wound contraction as compared to control. These effects were reversed when treated with ethanolic extract of *Ficus hispida.*

The dexamethasone-treated animal showed depressed wound healing, as evidenced by significant decrease in breaking strength, wet and dry tissue weight of granulation tissue, hydroxyproline and granulation tissue strength. In the same wound model, administration of *Ficus hispida* extract reversed the antihealing effect of dexamethasone. (Udupa, 2006).

CONCLUSION

The present study reveals that the standardized extract of *Ficus hispida* possesses good wound healing properties which may be attributed to the individual or combined action of phyto constituents like tannins, alkaloids and saponins present in it. Further investigations are necessary to determine the bioactive constituents present in the extracts used for studies.

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