

PROTECTIVE EFFECT OF DUMASIA VILLOSA FOR ITS WOUND HEALING ACTIVITY

Esha Yadav¹ & Ashoke K Ghosh²

Assistant Professor, Pharmacy Department, Kanpur Institute of Technology & Pharmacy,
Rooma, Kanpur, Uttar Pradesh, India.

Professor, Pharmacy Department, School of Pharmaceutical sciences, IFTM, University,
Lodhipur Road, Moradabad, , Uttar Pradesh, India .

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ABSTRACT: *Dumasia villosa* is a climbing fern it is the sole genus in the family fabaceae in Thailand its habitat is in abundance. It is used in external applications for rheumatism, sprains, scabies, eczema and cut wounds, they are reported to be particularly useful for carbuncles, reduce inflammation and acts as panacea for wounds, treat ulcer, various respiratory diseases, general disorders, muscles sprains. Leaves of *Dumasia villosa* is an important ingredient in most of the ayurvedic preparations.

The aim of the present study was to investigate the claimed tribal people's usage of leaves by using in vivo models for wound healing activity. The objective of present study is "Development of semisolid herbal formulation and its wound healing activity". Which posses maximum efficacy, reversibility of action, free from side effect and easy to use.

Key Words: Wound Healing, Excision model, Formulation, Ointment, Extract

I. INTRODUCTION

Herbal medicine has become an integral part of standard healthcare, based on a combination of time honored traditional usage and ongoing scientific research. Burgeoning interest in medicinal herbs has increased scientific scrutiny of their therapeutic potential and safety. Some of the medicinal plants are believed to enhance the natural resistance of the body to infections (Atal, C.K., 1986). Herbal preparation can be more effective and safer than conventional medicines. Non-toxic could be administered for a long period. Today's medicine is based on traditional medicines.

Wound healing is the process of repair that follows injury to the skin and other soft tissues. Following injury, are inflammatory responses occurs and the cells below the dermis (the deepest skin layer) begin to increase collagen (connective tissues) production. Later, the epithelial tissue (outer skin layer) is regenerated. There are three stages to the process of wound healing: inflammation, proliferation and remodeling. The proliferative stage is characterized by angiogenesis, collagen deposition, epithelialisation and wound contraction.

II. Material and methods

COLLECTION AND AUTHENTICATION OF PLANT MATERIAL

The plant of *Dumasia villosa* was collected in February from Dehradun region (Uttarakhand) and authenticated by **Tariq Hussain**, Scientist-in-charge, Raw material Herbarium and Museum, NBRI Lucknow, and a voucher specimen was also deposited (specimen number:98213). Leaves of *Dumasia villosa* were shade dried and powdered. An aliquot of powdered leaves (250 g) was submitted to successive solvent extractions with the help of soxhlet apparatus separately with petroleum ether, ethyl acetate, chloroform, ethyl alcohol and water. The extracts were evaporated to dryness in rota evaporator. Yields of each extracts were 6.8% for petroleum ether, 5.3% for chloroform, 5.3% for ethyl acetate, 24.0% for ethyl alcohol and 16.5% for water.

II. Animal

Albino rats which are weighing 200-220gm of either sex were taken from the animal house of University and kept in room at temperature $27 \pm 2^\circ\text{C}$, relative humidity 45-56%, light and dark cycles of 12 hrs each, during experiments. Animals were provided with standard rodent diet. Weight of animals was done before and after the experiment. The rats were prior anaesthetized during the experimental wound. The surgical operations were carried. All studies were performed by taking prior approval by the Institutional Animal

Care Committee, CPCSEA, India (Reg. No. 837/ac/04/CPCSEA). Chemicals used were of the analytical grade and the water used was always the double distilled water.

IV. Acute toxicity study

EDV at the dose level 2000mg/kg body weight of the animals was used for acute toxicity in accordance to Organization for Economic Cooperation Development guideline 423. Three male rats, each dosed at intervals of 48 hrs, were used. Mortality was determined over a period of 2 weeks.

V. Selection of doses

For determination of excision wound healing activity extract was formulated in ointment by using simple ointment as base. 5% and 10% (w/w) EDV ointments were applied where 500 mg and 1gm of EDV were used separately; in 10gms of ointment base. 0.5 g of EDV ointment and 0.3% nitrofurazone ointment was applied treat different groups of animals.

VI. Excision wound model

Animals were anaesthetized prior the creation of the wounds. The excision wounds were done as described by Morton and Malon (Morton et al., 1972). The fur of the animals were shaved with the help of an electric clipper and area of the wound to be created was outlined with the help methylene blue. The excision wound of 2 cm and 0.4cm in depth is created using toothed forceps, the entire wound was left open (Diwan et al., 1982). All animals were divided into 5 groups consisting of 6 animals each. Group one animals were treated with simple ointment. Group two, three and four were topically treated with the 4 percent EDV ointment, 5 percent EDV ointment and Group 5 0.2% treated with nitrofurazone ointment till complete healing. The closure rate of wound was done by tracing the wound on days 1, 4 and 9 post-wounding using transparent paper and permanent marker. The area of wound was measured by using a graph paper. Number of days required will give the the period of epithelization.

VII. Incision wound model

Rats were anaesthetized prior the creation of the wound. The dorsal fur of the animal was shaved with the help of an electric clipper. A longitudinal incision, six centimeters in length was made in the skin and in cutaneous on the back as described by the Ehrlich and Hunt et.al, 1976. After the incisions, sutures were applied to the skin at an interval of one centimeter. The wound was left undressed. The drugs was given orally to the different group, control and treated with EDV (100, 200, 400mg/kg) of body weight. The standard groups rats were treated with 0.2% nitrofurazone ointment. Sutures were removed on the 8th DAY post wound but the treatment remained continued. The skin-breaking strength was measured on the 10th DAY.

VIII. Dead space wound model

The animals were divided into 5 groups containing 6 each. Group 1 served as the control. Group 2, 3 and Group 4 were treated orally with EDV (suspended in 1% w/v CMC) of 100 mg/kg, 200mg/kg, 400mg/kg. Group 5 animals were treated with 0.2percent nitrofurazone ointment topically. Dead space wound model were inflicted by implanting two sterilized cotton pellets 10mg, one on either side in the lumbar region of the ventral surface of every rat. Then on the 10th post wound day, the granulation tissue which was formed on the surface of implanted cotton pellet was removed carefully. Then weight of the granulation tissue was noted. These granulation tissues were dried at 60°C for 12 hours, and the weight was recorded. Then to the dried tissue add 5ml 6N HCl and keep it at 110°C for 24hours. Then the neutralized acid of the dry tissue was used for the determination of hydroxyproline (Neuman et al., 1950).

IX. Determination of wound breaking strength

The anesthesia was given prior to the experiment and anaesthetized animal was secured at the table, and the draw a line on either side of the wound which should be 3 millimeter away from the line. The line was grapped with the help of forcep, one at each end opposit to each other. One forceps was supported firmly, and the other one was was connected to a freely suspended light weight metal plate. Weight were added slowly and then gradually weight is increased pulling apart the edges of wound. And as the wound gets open up, add on of weight was stopped and the weights which are added were noted down as the measure of breaking strength in grams (Shivhare et al., 2010)

X. RESULTS

Acute toxicity study

In this study, oral administration of EDV in male rats at 2000mg/kg had no effect on mortality, no clinical signs, no body weight changes are observed. Therefore, NO acute toxicity was found in rats treated with EDV and the approximate lethal doses for rats were determined to be higher than 2000mg/kg.

Table. 1 Wound healing effect of EDV in Excision wound model

| Parameter | Wound Area (millimeter square) on | | |
|-----------------|-----------------------------------|------------------------|------------------------|
| | Day 1 | Day 5 | Day 10 |
| Simple ointment | 441.50 ±0.42 | 371.40 ±0.96 | 200.82 ±1.34 |
| 4% EDV ointment | 436.83±.60** (3.26%) | 220.67±1.15** (40.43%) | 72.42±0.71*** (65.10%) |
| 5% EDV ointment | 449.17 ±0.48** (3.63%) | 150.76 ±0.67** (61.30) | 12.33 ±0.49*** (95.54) |
| Standard | 461.18±0.73** (3.97) | 120.834±0.75** (64.33) | 11.32±0.22*** (95.59) |

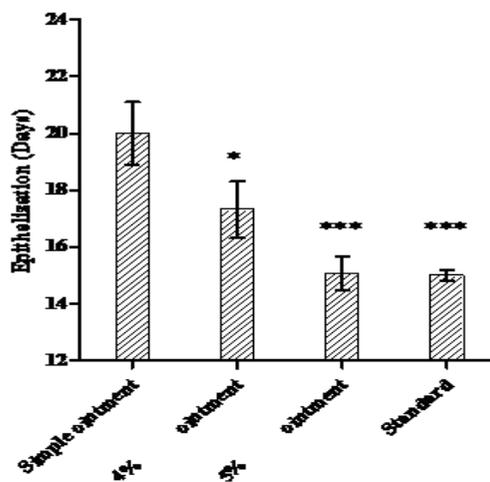


Fig. 1 Effects of the epithelization period of incision wound in simple and EDM ointment and Standard treated rats.

Table. 2 Wound healing effect of EDV in Incision wound model

| Parameter | Dose | Skin Breaking Strength (gm) |
|-----------|--------|-----------------------------|
| Control | 4ml/kg | 462 ± 5.62 |
| EDV | 100 | 472 ± 2.11* |
| EDV | 200 | 529 ± 3.62** |
| EDV | 400 | 563 ± 1.13*** |
| Standard | | 554 ± 1.12*** |

Values were expressed as mean ± SEM (n = 6).

Table.3 Wound healing effect of *D. villosa* in Dead space wound model

| Parameters | Dose (mg/kg) | Wet weight of the granulation tissue (mg/100g rat) | Dry weight of the granulation tissue (mg/100g rat) |
|------------|-----------------|--|--|
| Control | 4ml/kg | 92.95 ± 8.91 | 21.32 ± 9.10 |
| EDV100 | 100 | 99.50 ± 6.20* | 25.31 ±4.12* |
| EDV | 200 | 121.57 ± 5.35* | 24.58 ± 1.91** |
| EDV | 400 | 127.31 ± 4.15*** | 29.03± 1.22*** |
| Standard | | 135.30 ± 5.20*** | 30.40 ± 1.35*** |

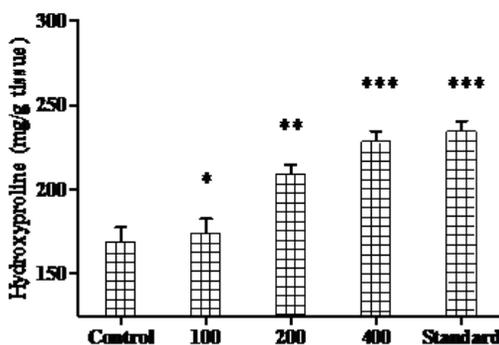


Fig. 2 Values in each bar is expressed as mean ± SEM (n = 6). in Dead space wound model. ANOVA followed by Dunnett test against control treated group. Significance represented as * (P<0.05), ** (P < 0.01), *** (P< 0.001).

Dead space wound

In the dead space wound study, there was a significant increase in dry weight of the granulation tissue of the treated group with EDV and was statistically significant (P<0.01). The dry weight of the granulation tissue of the group treated with 400mg/kg is comparable to the standard drug and were statistically significant (P<0.001) and shown in the table 3.

There was dose dependent increase in hydroxyproline groups of rats are treated with EDV 400mg/kg and standard drug treated rats were comparable and were statistically significant (P<0.001) as compared to control group rats and shown in the figure 3



Day 1 (Excision) Day 5 (Excision)



Day 10 (Excision)



Day 1 (Incision)



Day 5 (Incision)



Day 10 (Incision)

XI. DISCUSSION

Dumasia villosa is a climbing fern it is the sole genus in the family Fabaceae, . It occurs on mangrove and had tree dominated habitat sub division is petridophyte and had life form of cryptophytes category. It is commonly epiphytically grows on moss covered tree trunks, branches a lithophytes on shady boulders along with moss and in Thailand its habitat is in abundance. It is used in external applications for rheumatism, sprains, scabies, eczema and cut wounds, they are reported to be particularly useful for carbuncles, reduce inflammation and acts as panacea for wounds, treat ulcer, various respiratory diseases, general disorders, muscles sprains. In India it is found in Dehradun, Kumaon, Shahjanpur, Gorakhpur, throughout the plains in Bengal up to 5000 feet, both the sides of Madras state up to 40 00 feet and Kerela it is also found in Madhya Pradesh in regions like Annupur, Bastar, Betul, Bilaspur, Chhindwara, Damoh, Gwalior, Hoshangabad, Indore, Khandwa, Mandla, Raigarh, Raipur, Sidhi, Shivpuri and Panna. Lygodium is the climbing fern it is the genus of about 40 species native to tropical region across the world with a few temperate species in eastern Asia and eastern North America. Four species are recorded in India which are *D.circinatum*, *D.villosa*, *D.japonicum*, *D.microphyllum*. Traditionally *Dumasia villosa* was used as a medicine in many ways that I have studied in the literature survey for treating various diseases, so this plant has been selected for studies.

In pharmacological studies Wound healing activity was done and it was observed that topically administered drugs are effective in faster wound contraction due to the larger availability at the wound site. A significant increase in wound contraction was seen in both doses of EDV compared to control. Hence it appears that EDV has prohealing effect as evidenced by the above findings.

In incision wound, the increase in tensile strength of treated wounds may be due to the increase in both remodeling of collagen, and the formation of stable intra- and intermolecular crosslinks.

The collagen molecules synthesized are laid down at the wound site and become crosslinked to form fibres. Since incision wound treated with 4, 5% (w/w) SP showed greater tensile strength, it may be speculated that it not only increases collagen synthesis per cell, but also aids in cross-linking of the protein. The present study has demonstrated that an ethanol extract of *Dumasia villosa* leaves extract has properties that render it capable of promoting accelerated wound healing activity compared with the controls. Wound contraction increased tensile strength, increased hydroxyproline content.

Since EDV enhanced wound contraction, it would have either enhanced contractile property of myofibroblasts or increased the number of myofibroblasts recruited into the wound area. Granulation, collagen maturation and scar formation are some of the many phases of wound healing which run concurrently, but independent of each other. Topically administered drugs are effective in faster wound contraction due to the larger availability at the wound site. A significant increase in wound contraction was seen in both doses of EDV compared to control. Hence it appears that EDV has prohealing effect as evidenced by the above findings. In incision wound, the increase in tensile strength of treated wounds may be due to the increase in both remodeling of collagen, and the formation of stable intra- and intermolecular crosslinks. The collagen molecules synthesized are laid down at the wound site and become crosslinked to form fibres. Since incision wound treated with 4, 5% (w/w) SP showed greater tensile strength, it may be speculated that it not only increases collagen synthesis per cell, but also aids in cross-linking of the protein.

XII. Conclusion

The present study has demonstrated that an ethanol extract of *Dumasia villosa* leaves extract has properties that render it capable of promoting accelerated wound healing activity compared with the controls. Wound contraction increased tensile strength, increased hydroxyproline content.

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