WOUND HEALING ACTIVITY ON THE LEAVES OF ACHILLEA MILLEFOLIUM L. BY EXCISION, INCISION, AND DEAD SPACE MODEL ON ADULT WISTAR ALBINO RATS

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ABSTRACT
The aim of present study was to investigate the wound healing activity of the Indian medicinal plant Achillea millefolium L. The alcoholic and aqueous extracts of leaves of Achillea millefolium L. was studied for its effect on wound healing in Wistar rats, using incision, excision, and dead space model at dose levels of 200mg/kg. Alcoholic and aqueous extracts of leaves of Achillea millefolium L. showed a definite, positive effect on wound healing with a significant increase in the rate of wound contraction, skin breaking strength, granulation tissue dry weight and wet weight content, and breaking strength of granulation tissue. In histopathological studies showed increased collagen when compared to the control. The efficacy of Achillea millefolium L. in wound healing may be due to the presence of active principles, which accelerate the healing process and confers breaking strength to the healed wound. So it is possible to conclude that, this plant has wound healing activity and there by justifying traditional claim.

KEYWORDS: Achillea millefolium L.-asteraceae-excision-incision-dead space (NFZ)–tensiometer.

INTRODUCTION
Achillea millefolium L. is commonly known as yarrow belonging to the family asteraceae. Yarrow ethonopharmacologically used in folk medicine to treat inflammations, hemorrhoid1, wound healing, and dilate the blood vessels. The plant also has a long history as a powerful healing herb used topically for wounds, cuts and abrasions. Leaves of Achillea millefolium L. is alternate, sessile, linear to lanceolate, twice or ternately pinnatifid, Basal leaves to +25cm long. Cauleine leaves typically to +10cm long, +3cm broad. The main constituents2 of yarrow are isovaleric acid, salicylic acid, sterols, flavanoids, tannins, and coumarins.

The aim of the present study was to investigate the wound healing activity of leaves of Achillea millefolium L. in order to elucidate traditional3 use from the scientific point of view. The alcoholic and aqueous extracts prepared from the leaves of the mentioned plant were tested in rats for wound healing activity using excision, incision, and dead space wound models.

MATERIALS AND METHODS
Plant Material
The leaves of Achillea millefolium L. was collected, washed with water and dried. These were powdered by using electrical grinder and passed through sieve no #60 for powder analysis and the coarse fraction was subjected for phytochemical studies.

Extraction
The powdered leaves of Achillea millefolium L. was subjected to successive extraction with alcohol and aqueous separately on a continuous Soxhlet4 apparatus, filtered, solvent is reduced under pressure, and dried. The dried extracts were used for studies.

Animals
Wistar Albino rats of either sex, weighing about 150-250 g each were selected for these studies5. They were fed with food and water ad libitum and maintained in a cage under standard conditions (12 hour light-dark cycle; 35-60% humidity).

The experimental protocol was subjected to the surveillance of the Institutional Animal Ethics Committee. It was performed according to the international rules considering the animal experiments and biodiversity right. The rats were used after an acclimatization period of 7 days to the laboratory environment.

Acute Toxicity Studies
Adult Wistar Albino rats of either sex, fasted overnight, were divided into six groups with six animals in each group and were fed with increasing the doses 50, 100, 200, 500, 1000 mg/kg of alcoholic and aqueous extracts. The alcoholic and aqueous extracts administrated orally in doses of up to 2000 mg/kg of body weight.
No toxicity or death was observed for these given dose levels, in the selected and treated animals. So the LD$_{50}$ of the ethanolic and Aqueous extracts as per OECD guidelines-420 is greater than 2000mg/kg (LD$_{50}$>2000mg/kg). Hence the biological dose was fixed 100 and 200mg/kg for both the extracts.

**Wound Models**

The wound healing was carried out using ether-anaesthetized rats and their back was shaved in three different wound models such as excision, incision, and dead space models at dose levels of 200 mg/kg body weight.

**Formulations**

Two types of formulations were prepared form the extract viz 5% (w/w) and 10% (w/w) ointment were 5 g and 10g of extract were incorporated in 100g of simple ointment base BP. The extract ointment and simple ointment 0.5g each was applied once daily to treat different groups of animals, respectively nitrofurazone ointment (2% w/w, Smithkline- Beecham) was used as a standard drug for comparing the wound healing potential of the extract.

**Excision Wound Model**

Four groups with six animals in each group were anaesthetized with ether. The rats were depilated on the back. One excision wound was inflicted by cutting away a 500 mm full thickness of skin from the depilated area, the wound was left undressed to open environment. Then the drugs, i.e. the reference standard, (0.2% w/w NFZ) Nitrofurazone ointment, simple ointment BP, Achillea millefolium L. alcoholic extract ointment (10%w/w) and Achillea millefolium L. aqueous extract ointment (10%w/w) were administrated till the wound was completely healed. This model was used to monitor wound contraction and wound closure time. Wound contraction was calculated as percent reduction in wound area. The progressive changes in wound area were monitored plan metrically by tracing the wound margin on graph paper every alternate day (Fig I-VIII).

Statistical analysis data are expressed as mean +SEM and subjected to student’s t’ test by comparing with the control. (Tables and graph I-III)

**Dead Space Model**

Three groups of Wistar Albino rats (150-200g) were used. Dead space wounds were made by implanting, subcutaneously, a 2.5x 2.5 cm polypropylene tube beneath the dorsal paravertebral lumbar skin. Control animals received 2ml if 1% carboxy methyl cellulose (CMC), orally, which the test groups received Achillea millefolium L. extract 200mg/kg orally once daily for 10 days (Fig IX-X).

One the 11th post operative day, the granuloma tissue formed on the dead space wound was excised. Wet weight was recorded and the granuloma was dried in an oven at 60°C and dry weight was noted. (Tables and graph IV-V)

**Incision Wound Model**

Four groups with six animals in each group were anaesthetized and two paravertebral long incisions were made through the skin and cutaneous muscles at a distance of about 1.5 cm from the midline on each side of the depilated back of the rat. Full aseptic measures were not taken and no local or systemic antimicrobials were used throughout the experiments. Surgical threads (No. 000) and a curved needle (no.11) were used for stitching. The continuous threads on both wound edges were tightened for good closure of the wound. The wound was left undressed. All the groups were treated in the same manner as has already described above. They were administered once daily for 9 days. When wounds were cured completely the sutures were removed on the day 9 and tensile strength was measured with a tensiometer. (Fig XI-XIV& Tables and graph VI-VII)

**Histopathological Studies**

A section of the granuloma tissue was subjected to histopathological examination to determine the pattern of lay-down for collagen using Van Gieson and Masson Trichrome stains.

**Statistical Analysis**

Results, expressed as mean ± SE, were evaluated using one-way ANOVA with posthoc Scheffe’s post hoc test, Values of p <0.000 was considered statistically significant.

**RESULTS AND DISCUSSION**

The powdered leaves of Achillea millefolium L. belonging to family ‘Asteraceae’ was selected on the basis of ethanobotanical information and easy availability. So we validate scientifically for folk claim for its therapeutic activity we have also investigated in a systematic way covering pharmacological aspects in an attempt to rationalize its use as drug of therapeutic importance.

The process of wound healing occur in four phases: (I) coagulation, which prevents blood loss, (II) inflammation and debridement of wound, (III) repair, including cellular proliferation, and (IV) tissue remodeling and collagen position any agent, which accelerates the above process is a promoter of wound healing.

Plant products have been shown to possess good therapeutic potential of anticancer, antimicrobial and promoter of wound healing due to the presence of active
terpenes, alkaloids, and flavanoids. The wound healing property of *Achillea millefolium* L. appears to be due to the active principles which accelerates the healing process and confers breaking strengths to the healed wound.

In the all models studied, significantly improved wound-healing activity has been observed with the *Achillea millefolium* L. leaf extract compared to that of the reference standard and controls group animals. In excision wound model, it is observed that the wound contracting ability of the extract ointments were significantly greater than that of the control (p<0.01, p<0.001), which was comparable to that of the reference standard NFZ ointment. The extract ointment produced complete healing at 18th & 20th day by 10% w/w extract ointment respectively.

In the incision wound studies, there was a significant increase in tensile strength up to 510±14.6 (p<0.001) when compared with the control animal (415±18.2) significant increase in the weight of the granulation tissue (p<0.001).

In dead space wound model studies showed a significant increase in the wet granuloma tissue as well as in the dry weight. The tensile strength also found to be increased (p<0.001) in the extract treated groups and the level of hydroxyproline content.

**CONCLUSION**

The wound healing property of alcoholic and aqueous extracts of the leaves of *Achillea millefolium* L appears to be due to the presence of its active principles, which accelerates the wound healing process and confers breaking strength to the healed wound.

From the results obtained in the present investigation, it is possible to conclude that the leaves extract of *Achillea millefolium* L. has significant wound healing activity at the doses tested.

**ACKNOWLEDGEMENT**

The authors sincerely thanks to Principal Dr. K.L. Senthil kumar, chairman Padmavathi College of Pharmacy, Dharmapuri, Tamil Nadu, India for providing experimental facilities to carry out this work.

**REFERENCES**

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**Table 1: Effect of *Achillea millefolium* L. extract and Nitrofurazone on wound contraction in rats by excision model wound area (mm²)**

<table>
<thead>
<tr>
<th>Post wounding day</th>
<th>Simple ointment control</th>
<th>Nitrofurazone ointment (0.2% w/w)</th>
<th><em>Achillea millefolium</em> alcoholic extracts ointments (10% w/w each)</th>
<th><em>Achillea millefolium</em> aqueous extracts ointments (10% w/w each)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>524±34.6 (0.0)</td>
<td>518±27.2 (0.0)</td>
<td>529±33.4 (0.0)</td>
<td>534±39.2 (0.0)</td>
</tr>
<tr>
<td>2</td>
<td>509±26.1 (2.82)</td>
<td>479±39.4 (8.45)</td>
<td>478±25.2 (11.53)</td>
<td>429±26.4 (18.94)</td>
</tr>
<tr>
<td>4</td>
<td>484±21.4 (7.96)</td>
<td>340±34.2 (32.24)</td>
<td>352±19.5 (35.20)</td>
<td>335±21.5* (37.24)</td>
</tr>
<tr>
<td>6</td>
<td>425±17.2 (18.56)</td>
<td>275±21.1* (45.64)</td>
<td>276±17.4* (47.22)</td>
<td>217±16.4* (58.56)</td>
</tr>
<tr>
<td>8</td>
<td>381±16.9 (26.2)</td>
<td>186±16.4* (64.22)</td>
<td>198±15.5* (61.75)</td>
<td>114±11.7** (78.32)</td>
</tr>
<tr>
<td>10</td>
<td>320±13.4 (38.34)</td>
<td>125±9.8**(75.68)</td>
<td>118±10.4** (76.92)                                          64±5.6** (87.75)</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>279±11.5 (47.25)</td>
<td>72±5.4** (85.92)</td>
<td>82±6.4** (83.84)</td>
<td>36±2.9** (92.46)</td>
</tr>
<tr>
<td>14</td>
<td>241±10.3 (52.78)</td>
<td>52±3.7** (91.56)</td>
<td>56±4.7** (87.96)</td>
<td>18±1.4* (95.78)</td>
</tr>
<tr>
<td>16</td>
<td>205±9.5 (60.56)</td>
<td>38±2.5** (92.75)</td>
<td>35±2.4** (92.92)</td>
<td>5±0.6** (98.85)</td>
</tr>
<tr>
<td>18</td>
<td>156±7.2 (71.22)</td>
<td>12±1.4** (97.25)</td>
<td>14±0.8** (97.78)</td>
<td>0.0 (100)</td>
</tr>
<tr>
<td>20</td>
<td>129±9.1 (74.34)</td>
<td>0.0 (100)</td>
<td>0.0 (100)</td>
<td></td>
</tr>
</tbody>
</table>

P values versus respective control by student’s t test

*P< 0.01, **P< 0.001

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**Table 2: Dead Space Wound Model**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Treatment</th>
<th>Wet granuloma weight (mg)</th>
<th>Dry granuloma weight (mg)</th>
<th>Tensile strength</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (1% CMC, 2ml P.O)</td>
<td>225±6±15.2</td>
<td>33.6±2.8</td>
<td>383±21.4</td>
</tr>
<tr>
<td>2</td>
<td><em>Achillea millefolium</em> alcoholic extract (200mg/kg P.O)</td>
<td>460±24.5*</td>
<td>88.2±6.6*</td>
<td>528±38.4*</td>
</tr>
<tr>
<td>3</td>
<td><em>Achillea millefolium</em> aqueous extract (200mg/kg P.O)</td>
<td>508.54±32.6*</td>
<td>109.6±9.2*</td>
<td>614±46.2*</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± S.E., n=6, *P< 0.001 Vs control by students’ t test
Table 3: Incision Wound Model

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Tensile Strength</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Simple ointment (control)</td>
<td>415±18.2</td>
</tr>
<tr>
<td>2.</td>
<td>Alcoholic extract ointment (10% w/w)</td>
<td>510±14.6*</td>
</tr>
<tr>
<td>3.</td>
<td>Aqueous extract ointment (10% w/w)</td>
<td>548±25.5*</td>
</tr>
<tr>
<td>4.</td>
<td>NFZ ointment (0.2% w/w)</td>
<td>594±19.4*</td>
</tr>
</tbody>
</table>

Values are mean ± S.E of six animals in each group. Tensile strength was measure at the end of 9th day.

* P < 0.001 Vs respective control by students ‘t’ test.
Figure 3: Graphical representation of *Achillea millefolium* L. by dead space model
- Control (1% CMC, 2ms.P.O)
- *Achillea millefolium* alcoholic extract (200mg/kg PO)
- *Achillea millefolium* aqueous extract (200mg/kg PO)

Figure 4: Graphical representation of tensile strength of alcoholic and aqueous extracts of *Achillea millefolium* L. by incision wound model
1. Simple ointment, II. Alcoholic extract, III. Aqueous extract, IV. Nitrofurazone ointment

I. Group-1(Day 0) control

I. Group-2(Day 0) standard
III. Group-3 (Day 0) alcoholic extract

IV. Group-4 (Day 0) aqueous extract

V. Group-1 (Control) 16th Day

VI. Group-2 (Standard) 16th Day

VII. Group-3 (alcoholic extract 10%) 16th Day

VIII. Group-4 (Aqueous extract 10%) 16th Day

IX. Control (H&E 400×) showing deposited collagen fibers with minimum inflammatory cells.

X. Animals treated with test group (H&E 400×) showing collagen deposition and no inflammatory cells

XI. Incision model (Control) simple ointment

XII. Incision model (standard) nitrofurazone

XIII. Incision model (test) alcoholic extract

XIV. Incision model (test) aqueous extract