Healing Potential of Ferulic Acid on Dermal Wound in Diabetic Animals

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Abstract

The effect of Ferulic acid on wound healing activity is investigated in streptozotocin (50mg/kg) induced diabetic rats by excision, incision and dead space wound healing models in rats. Wistar albino rats of either sex weighing between 160-200 g were topically treated with Ferulic acid ointment by using simple ointment BP as base. 1 % (w/w) ointment was applied once daily in excision and incision wound healing model. Ferulic acid suspension was given orally at a dose of 40mg/kg in dead space wound healing models. Rats of standard groups were treated with 1% sofframycin ointment topically. The %wound closure, epithelization time, hydroxyproline content and tensile strength were measured. Topical application of Ferulic acid ointment in excision wound model increased the %wound closure. Epithelisaion period were decreased. In incision and dead space model wound breaking strength of wounds and hydroxyproline was increased. Ferulic acid accelerates wound healing in diabetic rats.

Key words: Diabetes, Excision wound, Incision wound, Wound contraction etc.

1. INTRODUCTION

Wound healing is a complex process that can be divided into at least 3 processes: an inflammatory reaction, a proliferative process, and tissue remodeling. Wound healing processes are strictly regulated by multiple growth factors and cytokines released at the wound site. Healing of wounds, a fundamental response to tissue injury occurs by a process of connective tissue repair. A fibrous scar is the end product of this process, the predominant constituent of which is collagen. Collagen and other components of the ground substance are synthesized by the highly vascular granulation tissue that is formed within the wound space. Since collagen provides strength and integrity to the dermis and all other supporting tissues, the synthesis, secretion and subsequent organization of collagen plays an integral role in wound healing. Diabetes mellitus is a condition which is known to be associated with a variety of connective tissue abnormalities. The collagen content of the skin is decreased as a result of reduced biosynthesis and/or accelerated degradation of newly synthesized collagen. Impaired wound healing is a significant problem in both Type 1 and Type 2 diabetes. Chronically increased
concentrations of blood glucose in diabetic patients, decreased concentration of growth factors, lead to impaired wound healing, decreased wound strength, and impaired wound-related angiogenesis [1-5]. Wound healing process in diabetic patient is impaired and delay due to high blood glucose level which decrease wound strength and impaired wound-related angiogenesis, and ferulic acid have anti-diabetic, anti-oxidant and angiogenic activity. Ferulic acid is reported to increase the level of vascular endothelial growth factor, platelet derived growth factor, hypoxia inducible factor-1α, but no such evidences are available which support its activity against wound healing in diabetic animal. So that the present study has been undertaken to examine the wound healing activity of ferulic acid in experimentally induced excision, incision and dead space wounds in diabetic rats [6-10].

2. MATERIALS AND METHODS
Ferulic acid (4-hydroxy-3-methoxycinnamic acid) is a phenolic compound found in plant tissues, and its constitutes a bioactive ingredient of many foods. Ferulic acid is found in seeds of plants such as in rice, wheat, and oats, as well as in coffee, apple, artichoke, peanuts, orange and pineapple.11

2.1. Experimental animals:
Male Wistar rats weighing 150–200 g were used for the study. The experimental rats were fed Commercial rat feed and water ad libitum.

2.2. Induction of diabetes:
Animals of group 2, 3 and 4 were weighed and their fasting blood glucose level were determined on zero day before inducing. The animals were then injected with a single dose streptozotocin (50 mg/kg body weight) in 0.1 M citrate buffer, pH 4.0 in the tail vein to induce diabetes. Control group were injected with 0.1M citrate buffer. Fasting blood glucose was measured 3 days later. Animals with glucose levels greater than 200 mg/dl were used for the study12.

2.3 Acute dermal toxicity (fixed dose procedure):
The acute dermal toxicity study was carried out in female albino rats by “fixed dose procedure” of OECD (Organization for economic co-operation and development) guideline no. 434. Ferulic acid was applied topically at a dose 2000 mg/kg.
The test substance was applied over an area which is approximately 10% of total body surface area with the help of a porous gauze dressing and non-irritating tape throughout a 24 hour exposure period. Then the animals were observed continuously for pain changes in skin eyes, mucous membrane, respiratory, circulatory, autonomic, central nervous systems, and behavior pattern, tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. Every 30 min for next 3 hour and finally for mortality after 24 hour till 14 days [13].

2.3. Grouping of animals:

For incision, excision and dead space wound model, 24 animals of either sex weighed between 150 and 200 g were divided into four groups in each model consisting of six animals as follows: group I - simple control group, group II - diabetic control and group III - drug treated and group IV standard treated was used.

2.4. Method of wound creation:

2.4.1 Excision wound model:

All animals in each group were anaesthetized with anesthetic ether before wound creation. An excision wound was inflicted by cutting away a 400mm² full thickness of skin from a predetermined area, the wound was left undressed to the open environment. Animals were closely observed for any infection and those which showed any sign of infection were separated, exclude from study and replaced. The standard drug ointment (1% soframycine), simple ointment base B.P. were applied topically to the standard group, ferulic acid ointment (1% w/w) base B.P. were applied to treated group , till the wound was completely healed. Wound areas were measured on days 0, 4, 8, 12, 16 for all groups, using transparency sheet and a permanent marker. Recording of wounds area were measured on graph paper. The day of scar falling, after wounding without any residual raw wound was considered as the day of epithelization. In this model wound contraction and wound closure time was monitored. Wound contraction was measured as percent contraction in each 4 days after wound formation [14].

2.4.2. Incision wound model:

All animals were anaesthetized before wound creation and one long incisions was made through the skin on depilated back of rat. All groups were treated same as in excision model, the both edges kept together and stitched with black silk surgical thread (no. 000) and a curved needle.
(no. 11) was used for stitching. The continuous threads on both wound edges were tightened for good closure of the wound. After stitching, wound was left undressed then, ferulic acid ointment and standard ointment were applied daily up to 9 days, when wounds were cured thoroughly the sutures were removed on the day 9 and tensile strength of cured wound skin was measured using tensiometer [15].

2.4.3. Dead space wound model:
This model was used for the study of granuloma tissue. Animals were anaesthetized by anesthetic ether and wound was made by implantation of two cotton plugs (10 mg each), one on either side, in the lumber region on the dorsal surface in each animal. On the ninth post-wounding day, granuloma tissue formed on an implanted cotton plug was dissected out carefully. Granuloma tissue from cotton plug was dried (60 °C) and stored in 10% formalin for the biochemical parameters [14].

2.5. Wound healing evaluation parameters:
2.5.1. Wound contraction and epithelialization time:
An excision wound margin was traced after wound creation by using transparent paper and area measured by graph paper. Wound contraction was measured in each 4 days interval, until complete wound healing and expressed in percentage of healed wound area. The epithelialization time was measured from initial day [16].

The percentage wound contraction was determined using the following formula:

\[
\text{Percent wound contraction} = \frac{\text{Healed area}}{\text{total wound area}} \times 100
\]

2.5.2. Measurement of tensile strength:
Tensile strength is the resistance to breaking under tension. It indicates how much the repaired tissue resists to breaking under tension and may indicate in part the quality of repaired tissue. Sutures were removed on the day 9 after wound creation and the tensile strength was measured. For this purpose, the newly formed tissue including scar was excised and tensile strength was measured with the help of tensiometer. In this method wound breaking strength was measured as the weight of water at the time of wound breaking per area of the specimen [14-15].
2.5.3. Hydroxyproline estimation:
Granuloma tissues were dried in a hot air oven at 60-70 °C to constant weight and were
hydrolyzed in 6N HCl at 130°C for 4 h in sealed tubes. The hydrolysate was neutralized to pH
7.0 and was subjected to Chloramine-T oxidation for 20 min. The reaction was terminated by
addition of 0.4M perchloric acid and color was developed with the help of Ehrlich reagent at 60
°C and measured at 557 nm using a spectrophotometer [17].

2.6. Statistical analysis:
Treated group was compared with the control group. The results were analyzed statistically using
tukey’s-test to identify the differences between the treated and control. The data were considered
significant at $P < 0.05$

3. RESULTS

3.1 Acute dermal toxicity:
Acute dermal toxicity on female rats showed no mortality at a dose of 2000 mg/kg, during time
period of 14 days. No skin allergic Symptoms were seen. The behavioral, neurological,
autonomic responses were studied for a time period of 6 hours of toxicity study. During the study
no noticeable response were seen in the rats.
None of the animal showed toxicity in acute dermal toxicity test. And the
parameters which are mention in irwins tables are also normal in all animals except piloerection
activity.

Table no. I  Acute dermal toxicity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose(mg/kg)</th>
<th>Number of animals</th>
<th>Mortality After 24 hrs</th>
<th>Mortality After 7 days</th>
<th>Mortality After 14 Days</th>
<th>Toxicity profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferulic acid</td>
<td>2000</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Safe</td>
</tr>
</tbody>
</table>
### Table no. II  Irwin’s Table

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Treatment</th>
<th>PARAMETER OBSERVED</th>
<th>[ \text{Review} ]</th>
<th>[ \text{Review} ]</th>
<th>[ \text{Review} ]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Behavioural Response</td>
<td>Neurological Response</td>
<td>Autonomic Response</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Ferulic acid</td>
<td>Alertness</td>
<td>Stereotypy</td>
<td>Irritability</td>
<td>Touch Response</td>
</tr>
<tr>
<td>1</td>
<td>Normal</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>a b</td>
</tr>
</tbody>
</table>

N= Normal
ab= absent

3.2. Tensile strength of incision wound:

Tensile strength for the treated group on day 10 was found to be significant \( P < 0.05 \) than normal and diabetic control group as shown in (Tables no.III) (Figure I, II) (Histogram I).

**Table No. III: Tensile Strength (gms)**

<table>
<thead>
<tr>
<th>Group I Normal control</th>
<th>Group II Diabetic control</th>
<th>Group III Diabetic standard</th>
<th>Group IV Drug treated (ferulic acid ointment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>49.17±3.055</td>
<td>30.06±2.505*</td>
<td>167.8±6.301***</td>
<td>97.47±6.358***</td>
</tr>
</tbody>
</table>
3.3. Wound contraction:

Wound area was measured by tracing the wound margin using a transparent paper in each 4 days interval and healed area calculated by subtracting from the original wound area. On day 4, the wound contraction of standard and ferulic acid ointment treated groups was found to be significant ($P < 0.05$) in comparison to normal and diabetic control group. On day 16, standard ointment treated wound was almost completely healed (Table no.IV,V). On day 18, ferulic acid treated group healed 100% but normal and diabetic treated group not completely healed. It was also observed that epithelialization period of treated and standard group were less in comparison to simple ointment base treated group.

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### Table no. IV: Wound area (mm²)

<table>
<thead>
<tr>
<th>Group</th>
<th>Normal control</th>
<th>Diabetic control</th>
<th>Diabetic standard</th>
<th>Drug treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>370.6±13.07</td>
<td>345.2±3.513</td>
<td>346.2±9.972</td>
<td>375.0±13.60</td>
</tr>
<tr>
<td>4</td>
<td>286.2±16.12</td>
<td>296.8±5.908&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>235.6±7.646**</td>
<td>273.2±11.03&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>8</td>
<td>190.8±8.924</td>
<td>209.4±6.683&lt;sup&gt;***&lt;/sup&gt;</td>
<td>88.00±3.368&lt;sup&gt;***&lt;/sup&gt;</td>
<td>124.6±8.232*</td>
</tr>
<tr>
<td>12</td>
<td>80.40±5.767</td>
<td>149.0±4.343&lt;sup&gt;***&lt;/sup&gt;</td>
<td>29.80±2.354&lt;sup&gt;***&lt;/sup&gt;</td>
<td>53.40±5.250*</td>
</tr>
<tr>
<td>16</td>
<td>35.80±3.338</td>
<td>59.40±4.343&lt;sup&gt;***&lt;/sup&gt;</td>
<td>8.00±1.304&lt;sup&gt;***&lt;/sup&gt;</td>
<td>14.40±1.364&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

### Table no. V: Percentage wound closure

<table>
<thead>
<tr>
<th>Group</th>
<th>Normal control</th>
<th>Diabetic control</th>
<th>Diabetic standard</th>
<th>Drug treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>22.79%</td>
<td>14.02%</td>
<td>31.94%</td>
<td>27.21%</td>
</tr>
<tr>
<td>8</td>
<td>48.61%</td>
<td>39.04%</td>
<td>74.91%</td>
<td>66.84%</td>
</tr>
<tr>
<td>12</td>
<td>78.34%</td>
<td>56.14%</td>
<td>91.32%</td>
<td>87.27%</td>
</tr>
<tr>
<td>16</td>
<td>90.89%</td>
<td>82.77%</td>
<td>97.78%</td>
<td>96.28%</td>
</tr>
</tbody>
</table>

### Table no. VI: Period of Epithelisaion in days

<table>
<thead>
<tr>
<th>Normal control</th>
<th>Diabetic control</th>
<th>Diabetic standard</th>
<th>Drug treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>22±0.447</td>
<td>27±0.316&lt;sup&gt;***&lt;/sup&gt;</td>
<td>17±0.372&lt;sup&gt;***&lt;/sup&gt;</td>
<td>20±0.447&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Figure-III Excision wound: 0 day status

(a) Control group (b) Diabetic control (c) Standard treated (d) Drug treated
Figure IV  Excision wound: 16 day status

(a) Control group (b) Diabetic control (c) Standard treated (d) Drug treated
0 day wound status

Histogram II: Zero day wound area.

4th day wound status

Histogram III: 4th day wound area.

8th day wound status

Histogram IV: 8th day wound area.

12th day wound status

Histogram V: 12th day wound area

16th day wound status

Histogram VI: 16th day wound area.
3.4. Granuloma weight and Hydroxyproline:
Granuloma weight of treated animal groups was found to be increased when compared with control group. Treated group showed significant increased hydroxyproline level when compared to control group ($P < 0.05$) in (Tables no. VII) (Figure V) (Histogram VIII, IX).

Table no. VII: Wound parameter

<table>
<thead>
<tr>
<th>Groups</th>
<th>Normal control</th>
<th>Diabetic control</th>
<th>Drug treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet granulation (mg)</td>
<td>123.3±39.30</td>
<td>130.0±20.82</td>
<td>226.7±31.80**</td>
</tr>
<tr>
<td>Dry granulation (mg)</td>
<td>49.33±12.02</td>
<td>33.33±3.333</td>
<td>56.67±8.819**</td>
</tr>
<tr>
<td>Hydroxyproline (µg/ml)</td>
<td>4.76±0.523</td>
<td>3.76±0.50</td>
<td>9.16±0.348***</td>
</tr>
</tbody>
</table>

Histogram VIII: Comparison of wet and dry granulation weight in all 3 groups
Figure-V: Standard curve of hydroxyproline.

Histogram IX: Comparisons of hydroxyproline in all 3 groups.

4. DISCUSSION

Diabetes mellitus is known to be associated with a variety of alterations in connective tissue metabolism, as a result of which diabetics face the problem of poor wound healing [2]. Collagen is a major and abundant protein of the extracellular matrix and provide strength to wounds [18]. Loss of collagen observed in diabetes may be due to decreased levels of synthesis or enhanced catabolism of newly synthesized collagen, or both [2]. As ferulic acid was reported to cause hypoglycemic effects. It was felt that it would be interesting to study its influence on the healing
of wounds in diabetic conditions. Results obtained in the present study suggest that treatment of diabetic rats with ferulic acid may have a beneficial influence on wound healing.

Wound healing process is a complex process of the body’s immune system. It is a part of homeostatic mechanism it can be divided into 3 phases: inflammatory phase, proliferation phases and remodeling. This wound healing process is triggered by release of various molecule like vascular endothelial growth factor (VEGF) [9], platelet derived growth factor (PDGF) [10], tissue growth factor (TGF-α, TGF-β), epithelial growth factor (EGF), insulin growth factor-1 (IGF-1) and very important hypoxia induced growth factor (HIF-1) [8].

This wound healing process in diabetic patient is impaired and delay due to high blood glucose level which decrease wound strength and impaired wound-related angiogenesis [5]. This alteration occurs due to advance glycation end product which causes protein glycation, Protein glycation and AGE (advanced glycation product) are accompanied by increased free radical activity that contributes towards the biomolecular damage. These AGEs also causes atherosclerosis which may lead to diminish vascularisation and finally impaired wound healing process.

In incision wound model the ferulic acid treated animal showed greater wound tensile strength. The higher tensile strength may be due to higher amount of collagen synthesis and increased cross linking between newly developed tissue and cells. The ferulic acid proved to increase ulcer healing and cell proliferation [19] by investigator. Hence there may be chances with ferulic acid to increase tensile strength by antioxidant [20] ulcer healing and proliferating properties.

In excision wound model ferulic acid treated group showed more wound contraction and enhanced epithelization as compared to normal and diabetic group. In dead space model, there was also significant increase in hydroxyproline content due to greater deposition of collagen because hydroxyproline is the constituent of collagen. Ferulic acid treated group which is the major constituent of collagen. Increase in dry granuloma weight indicated higher protein content [15].

Therefore on the basis of above result we stat that ferulic acid promotes healing of wounds, by enhancing rate of collagen turnover, period of epithelization and wound contraction in streptozotocin induced diabetic rats.
5) References:


13. OECD guideline for testing of chemicals proposal for a new draft guideline 434: acute dermal toxicity – fixed dose proceduredraft guideline ,14 may 2004 (1st version).


