thick glorious branch lets. The tree has a straight trunk and grey or reddish bark, masked by large white patches. It has green leaves with a length and width of 6 to 15 cm, with 5 to 7 shallow lobes. The branches contain whitish latex, which causes brown stains. Inflorescences are formed terminally on branches. The plant is monoecious and flowers are unisexual.\[4-5\] After pollination, a trilocular ellipsoidal fruit is formed. The seeds are black and in the average 18 mm long and 10 mm wide ripe Jatropha fruits.\[6\] It is a multipurpose species with many attributes and considerable potential. The wood and fruit of Jatropha can be used for numerous purposes including fuel. It is used against dermatomucosal diseases, arthritis, gout, jaundice, Toothache, gum inflammation, gum bleeding, diarrhoea and pyorrhea.\[7\] Plant extract used to treat Allergies, burns, cuts and wounds, inflammation, leprosy, leucoderma, scabies and small pox. Water extract of branches used in HIV, tumor and Wound healing. The plant contains Organic acids, Cyclic triterpenes stigmasterol,\[8\] Curcacycline A, Curcin,\[9\] a lectin Phorbolesters Esterases, Sitosterol and its d-glucoside.\[10\] The leaf and bark have been shown to contain glycosides, tannins, phytoestrogens, flavanoids and steroidal sapogenins.\[7\]

The plant is reported to have properties against diseases. In view of these cited activities, observations and traditional uses of plant, the present study was undertaken to explore the wound healing potential of extract of this plant in excision and incision experimental models.

Wound Healing Potential of Extract of <i>Jatropha curcas</i> L. (Stem bark) in rats

Kamal Sachdeva*, Preeti Garg,1 Manmohan Singhal,2 Birendra Srivastava2

1School of Pharmacy, Suresh Gyan Vihar University, Jaipur, India. 2School of Pharmaceutical sciences, Jaipur National University, Jaipur, India

ABSTRACT

Introduction: The present study provides a scientific evaluation for the wound healing potential of extract of <i>Jatropha curcas</i> L. stem bark. <i>Jatropha curcas</i> L. or physic nut, is a bush or small tree (up to 5 m height) and belongs to the Euphorbiaceae family. Methods: Excision and incision wounds were inflicted upon four groups of six rats each. Group I was assigned as control (ointment base), Group II was treated with standard silver sulfadiazine (0.01%) cream, Group III and Group IV was treated with 5% and 10% extract ointment respectively. The parameters observed were percentage of wound contraction, hydroxyproline content and tensile strength including histopathological studies. Results: It was noted that the effect produced by the extract ointment showed significant (\(P < 0.01\)) healing in both the wound models when compared with control group. All parameters such as wound contraction, hydroxyproline content, tensile strength and histopathological studies showed significant changes when compared to control. Conclusion: The result shows that extract ointment demonstrates wound healing potential in both excision and incision models.

Key words: Histopathological, Hydroxyproline, Euphorbiaceae, sulfadiazine, tensile

INTRODUCTION

Herbal medicines have been enjoying revitalization among the clients all over the world. There are hundreds of medicinal plants that have a long history of curative properties against various diseases and ailments. However, screening of plants for their activity is very crucial and needs imperative attention in order to know the value of the plant. The assessment of the plants for their therapeutic activity is done on the basis of either their chemotaxonomic examination or ethnobotanical information for a particular disease.\[1\]

<i>Jatropha curcas</i> L. or physic nut, is a bush or small tree (up to 5 m height) and belongs to the Euphorbiaceae family and contains approximately 170 known species.\[2\] <i>Jatropha</i>, a drought-resistant shrub or tree, which is widely distributed in the wild or semi-cultivated areas in Central and South America, Africa, India and South East Asia.\[3\] It is a multipurpose, drought resistant, perennial plant gaining lot of importance for the production of biodiesel. It has
MATERIAL AND METHODS

Plant material
Fresh stem bark of *Jatropha curcas* L. was collected from a local area of Jaipur and were identified in the department of botany, Rajasthan University, Jaipur. A voucher specimen number RUBL20844 was deposited in the department. The fresh stem bark was air-dried to constant weight, pulverized and stored in an air-tight container for further use.

Extraction of plant drug
Powder of dried stem bark was subjected to soxhlet extraction with methanol: acetone: water (70:20:10). The extract was then filtered and the filtrate was concentrated to dryness.

Preliminary Phytochemical Screening
The extract was subjected to phytochemical tests for tannins, steroids, alkaloids and glycosides, flavonoids, carbohydrates, proteins and amino acid using reported methods.[11-12]

Preparation of formulation and standard used
5% (w/w) & 10% (w/w) simple ointment containing the extract of plant was prepared by trituration method in a ceramic mortar and pestle using white soft paraffin base. For this, 5 g & 10 g extract was incorporated in 100 g of the base. Silver sulfadiazine (0.01%) obtained from Rexin Pharmaceutical Pvt. Ltd. was used as standard drug for comparing the wound healing potential of extract in different animal models.

Animals
Albino rats of either sex (150-200 g) were used for the experimental study. The animals obtained from Shree Dhanvantary Pharmaceutical Analysis and Research Centre, Kim, Surat were maintained under standard husbandry conditions in polypropylene cages and provided with food and water ad libitum. The animals were kept on fasting overnight prior to the experimentation and all the procedures used in these studies were approved by the Institutional Animal Ethics Committee.

Grouping of animals
Four groups of animals containing six in each were used for excision and incision wound models. The animals of groups I, II, III and IV were considered as the control, reference standard, (5%) & (10%) extract ointment respectively.

In vivo studies
Excision wound model
The animals were divided into three groups with six in each were anaesthetized by open mask method with anesthetic ether before wound creation. The particular skin area was shaved 1 day prior to the experiment. An excision wound was inflicted by cutting away a 300 mm² full thickness of skin from a predetermined shaved area.[13] The wounds were left undressed to the open environment. The ointment base, standard drug ointment (0.1% silver sulfadiazine) and extract of plant ointment (5%, w/w) & (10%, w/w) were applied topically to the control group, standard group and treated group respectively, till the wound was completely healed. In this model, wound contraction was monitored. Wound contraction was measured as percent contraction in each 2 days after wound formation. From the healed wound, a specimen sample of tissue was collected from each rat for histopathological examination.[14-15]

Incision wound model
In incision wound model,[16] all the animals of each group were anaesthetized under light ether anesthesia. Two full thickness paravertebral long incisions were made through the skin at the distance of about 1 cm from midline on each side of the depilated back of rat. After the incision was made the both edges of skin 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 ointment base, standard ointment and extracts ointment were applied daily up to 10 days; when wounds were cured thoroughly the sutures were removed on the day 10 and tensile strength of cured wound skin was measured using tensiometer.[17]

WOUND HEALING EVALUATION PARAMETERS

Measurement of wound contraction
An excision wound margin was traced by following the progressive changes in wound area planimetrically, excluding the day of wounding. The size of wounds was traced on a transparent paper in every 2 days, throughout the monitoring period. The tracing was then shifted to graph paper, from which the wound surface area was evaluated.[18] The evaluated surface area was then employed to calculate the percentage of wound contraction, taking initial size of wound, 300 mm², as 100%, by using the following formula as:

\[
\text{% wound contraction} = \frac{\text{initial wound size} - \text{specific day wound size}}{\text{initial wound size}} \times 100
\]

Measurement of tensile strength
The force required to open the healing action is known as tensile strength. It is used to measure the completeness of healing. It also indicates how much the repaired tissue resists to breaking under tension and may indicate in part the quality of repaired tissue. The sutures were removed...
on the 9th day after wounding and the tensile strength was measured on 10th day. For this purpose, the newly formed tissue including scar was excised and tensile strength was measured with the help of tensiometer.[19] In this method, wound-breaking strength was measured as the weight of water at the time of wound breaking per area of the specimen.

Hydroxyproline estimation
Hydroxyproline is an uncommon amino acid present in the collagen fibres of granulation tissues. Its estimation helps clinically to understand progress rate at which the healing process is going on in the connective tissue of the wound. For the determination of hydroxyproline content, the wound tissues were excised and dried in a hot air oven at 60-70 °C to constant weight and were hydrolysed in 6N HCl at 130 °C for 4 h in sealed glass 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. The absorbance was measured at 557 nm using a spectrophotometer. The amount of hydroxyproline in the samples was calculated using a standard curve prepared with pure l-hydroxyproline.[20]

Histopathological examinations
A specimen sample of skin tissues from control, standard and treated groups was taken out from the healed wounds of the animals in excision and incision wound models for histopathological examinations. The thin sections were cut and stained with haematoxylin and eosin [21] and observed under microscope for the histopathological changes such as fibroblast proliferation, collagen formation and angiogenesis.

Table 1: Effect of methanolic extract of *Jatropha curcas* L. and standard ointment on % of wound contraction of excision wound models in rats

<table>
<thead>
<tr>
<th>Post wounding days</th>
<th>% of wound contraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
</tr>
<tr>
<td>2</td>
<td>8.72 ± 1.791%</td>
</tr>
<tr>
<td>4</td>
<td>19.13 ± 1.528%</td>
</tr>
<tr>
<td>6</td>
<td>30.55 ± 3.055%</td>
</tr>
<tr>
<td>8</td>
<td>40.47 ± 2.107%</td>
</tr>
<tr>
<td>10</td>
<td>47.79 ± 1.51%</td>
</tr>
<tr>
<td>12</td>
<td>55.21 ± 2.473%</td>
</tr>
<tr>
<td>14</td>
<td>66.70 ± 2.91%</td>
</tr>
<tr>
<td>16</td>
<td>74.12 ± 3.276%</td>
</tr>
<tr>
<td>18</td>
<td>83.41 ± 3.602%</td>
</tr>
<tr>
<td>20</td>
<td>89.95 ± 1.67%</td>
</tr>
<tr>
<td>22</td>
<td>93.64 ± 4.71%</td>
</tr>
<tr>
<td>24</td>
<td>97.38 ± 1.82%</td>
</tr>
<tr>
<td>26</td>
<td>99.91 ± 5.98%</td>
</tr>
</tbody>
</table>
DISCUSSION AND CONCLUSION

Wound healing is a stepwise process, which consists of different phases such as hemostasis, inflammation, proliferative and remodeling or maturation. The genetic response regulating the body’s own cellular resistance mechanisms contributes to the wound and its repair.\textsuperscript{[22]} Hence in this study, excision and incision wound models were used to evaluate the effect of extract ointment on various phases.

In incision wound, the increase in tensile strength of treated wounds may be due to the increase in collagen concentration and stabilization of the fibres.\textsuperscript{[23]} Increase in blood vessels and role of antioxidants were experimentally proved.\textsuperscript{[24]} In excision wound, the extract showed faster healing with earlier wound contraction compared with control groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hydroxyproline (mg/g tissue)</th>
<th>Tensile strength (g/mm\textsuperscript{2})</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>25.76 ± 0.003</td>
<td>413.80 ± 3.665</td>
</tr>
<tr>
<td>standard</td>
<td>61.52 ± 0.004*</td>
<td>607.22 ± 3.717*</td>
</tr>
<tr>
<td>Extract ointment (5%)</td>
<td>43.39 ± 0.002*</td>
<td>493.75 ± 4.136*</td>
</tr>
<tr>
<td>Extract ointment (10%)</td>
<td>55.76 ± 0.003*</td>
<td>582.80 ± 3.665*</td>
</tr>
</tbody>
</table>

\(n = 6\) albino rats per group; values represents mean ± SEM. \(*P < 0.01\) (comparison of control with standard & extracts).

Figure 1: Histopathological characteristics of rat skin on 18th day by treatment with 5% Extract ointment

Figure 2: Histopathological characteristics of rat skin on 18th day by treatment with 10% Extract ointment

Figure 3: Histopathological characteristics of rat skin on 18th day by treatment with ointment base

Figure 4: Histopathological characteristics of rat skin on 18th day by treatment with Standard ointment

Collagen fibres and blood vessels are prominently present in standard and extract treated group as compared to control.
The earlier wound contraction rate of the extract may be due to stimulation of interleukin-8, an inflammatory a-chemokine which affects the function and recruitment of various inflammatory cells, fibroblasts and keratinocytes. It may increase the gap junctional intracellular communication in cultured fibroblasts and induces a more rapid maturation of granulation tissue.[25] The extract of plant increased cellular proliferation and collagen synthesis at the wound site as evidenced by increase in total protein and total collagen contents reflected by hydroxyproline content of granulation tissues. The glycosaminoglycans are a major component of the extra cellular matrix of skin, joints, eyes and many other tissues and organs. In spite of its simple structure, it demonstrates remarkable visco-elastic and hygroscopic properties which are relevant for dermal tissue function. Biological activities in skin are due to its interaction with various binding proteins. Due to an influence on signaling pathways, hyaluronic acid and hydroxyproline is involved in the wound-healing process and scarless fetal healing. In clinical trials, topical application of hyaluronic acid has improved the healing of wound.[26] In addition, the muco-polysaccharide hyaluronic acid protects granulation tissue from oxygen free radical damage and thereby stimulates wound healing.[27] Among the glycosaminoglycans, hydroxyproline, dermanan sulfate and dermanan have also been implicated in wound repair and fibrosis. Their ability to bind and alter protein–protein interactions has identified them as important determinants of cellular responsiveness in development, homeostasis and disease.[28]

The results showed that extract ointment possesses a distinct prohealing stroke. This was demonstrated by a significant increase in the rate of wound contraction. Significant increase ($P < 0.01$) in tensile strength, and hydroxyproline content were observed, which was auxiliary supported by histopathological studies. This indicated newly formed fibroblasts cells, collagen fibres and blood vessels. Recent studies with other plant extracts have shown that phytochemical constituents like flavanoids,[29] triterpenoids[30] and tannins[31] are known to promote the wound-healing process.

Preliminary phytochemical screening of extract of *Jatropha* showed the presence of alkaloids, flavonoids and tannins. Its chemical constituents mainly consist of oils and fats, org. acids, flavonoids, triterpenes, steroids, sterols, and proteins. The wound healing action of *Jatropha* may probably be due to the phytoconstituents present in the plant or could be a function of either the individual or the additive effects of the phytoconstituents.

Hence, the results obtained from data concludes that extract ointment of plant has properties that render it capable of promoting wound healing activities such as stimulating wound contraction and increasing tensile strength of incision as compared to control.

**ACKNOWLEDGEMENTS**

The authors are thankful to Jaipur National University, Jaipur for providing facilities to carry out this work and department of botany, Rajasthan University, Jaipur for authentication of plant.

**REFERENCES**


Pharmacognosy Journal | September 2011 | Vol 3 | Issue 25

71