An assessment of the wound healing potential of a herbal gel containing an *Azadirachta indica* leaf extract

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Abstract: The objective of this study was to produce a Carbopol 940 based gel formula containing an *Azadirachta indica* leaf extract and evaluate its wound healing potential. The ethanolic extract was derived from the dried leaves of *Azadirachta indica* and was subjected to a phytochemical evaluation. Three gel formulations of Carbopol 940 containing an *Azadirachta indica* extract in three different concentrations, i.e., 1, 2, and 3% w/w were prepared. These gels were evaluated for their physical appearance, stability, antimicrobial activity, extrudability, skin irritability, pH, spreadability, and viscosity. The prepared formulas were stable, greenish and homogeneous. None of them showed irritation to the skin. The spreadability (g·cm/sec), viscosity (cps), and pH of all three formulations was 34.68, 53 270–65 400, and 6–7, respectively. Gel-III exhibited the highest antimicrobial potential against *E. coli* and *P. aeruginosa* with a zone of inhibition of 16.2 ± 0.6 mm and 15.6 ± 0.6 mm, respectively. It was revealed from the wound healing studies that the epithelialisation time for the Albino rabbits treated with Gel-III was 23 days. The Albino rabbits treated with Gel-I, Gel-II, a standard gel, and those with the untreated one (control) epithelialised in 27, 25, 26, and 34 days, respectively. A formulation containing 3% w/w extract showed better antimicrobial activity, physicochemical characteristics, and pharmacological parameters than the other formulations. It can be concluded that the wound healing process was faster with the gel formulation containing 3% w/w of the *Azadirachta indica* extract, proposing that this formulation is a promising candidate for wound healing.

Keywords: antimicrobial activity; Carbopol 940; epithelialisation time; ethanolic extract; phytochemical evaluation

A wound is a physical injury that results in the breaking of the functional and cellular continuity of cells and can be created by microbial, biological, physical, or chemical disturbances to the skin (Ayello 2005; Sarimah and Mizaton 2018). Wound healing occurs in a complex, organised, and dynamic mode that involves replacing the devitalised structures of cells and tissues and occurs in four phases, i.e.,
haemostasis, inflammation, proliferation, and maturation (also named remodelling). Natural wound healing can take several days or weeks and wounds are very prone to bacterial infections (Sarimah and Mizaton 2018). Various topical formulations such as ointments, gels, or wound dressings are available to protect the wound from disease and accelerate the wound healing. Gels are simple to apply to the wounds and can be washed easily. Gels are promising drug delivery tools, especially for topical treatments.

Herbal remedies are traditionally used for wound healing all over the world (Yang et al. 2019; Gao et al. 2020). According to the World Health Organization, more than 80% of individuals in developed countries consume natural products (Gupta et al. 2013). Several studies have been carried out in developing countries like China and India (Tong et al. 2019; Chen 2020), where mostly wild plants are used to treat burns and wounds (Krishnan 2006; Kumar et al. 2007). Herbs have been used for years as folk medicines (Zhao et al. 2020) because of the lower side effects than modern pharmaceuticals and synthetic drugs (Joshi et al. 2011).

Azadirachta indica (family Meliaceae) is a medicinal plant, commonly known as neem, found in India, Pakistan, Bangladesh, and Nepal, and is widely used to treat various diseases (Parrotta and Chaturvedi 1994; Puri 1999; Biswas et al. 2002). Neem has been commonly used in Ayurveda, Unani, Homoeopathic, and Siddha medicine and has become prominent in modern medicine (Maithani et al. 2011). The limonoids and azadirachtin found in neem seeds have insecticidal effects, but are safe for human beings. The leaf extract, seed oil, and bark of the neem are medicinally used in folk medicine for constipation, respiratory disorders, leprosy, and intestinal helminthiasis and they promote good health. Neem also possesses antipyretic, anti-inflammatory, antymycotic, antimicrobial, immunomodulatory, cardiovascular, anti-hyperglycaemic, and neuropsychological activities. All parts of the neem plants are used to treat itching, burning sensations, blood morbidity, and skin ulcers (Barua et al. 2010). Steroids, alkaloids, flavonoids, fatty acids, carbohydrates, and terpenoids (Saleem et al. 2018) are some of the various phytochemicals found in Azadirachta indica. The acetone and water extract of Azadirachta indica possess antimicrobial activities against Pseudomonas aeruginosa and Staphylococcus aureus (Osunwoke Emeka et al. 2013).

In this study, gel formulations containing Carbopol 940 and an extract of Azadirachta indica were prepared to investigate the activity of wound healing. Gels are popular because of the ease in their application and improved percutaneous absorption compared to other preparations.

MATERIAL AND METHODS

Materials

Carbopol 940, methylparaben, propylparaben, triethanolamine, and propylene glycol were obtained from Merck (Darmstadt, Germany). The polyethylene glycol was obtained from Fluka (Steinheim, Germany).

Plant materials and preparation of extract

Azadirachta indica leaves were collected, cleaned from foreign material, washed with distilled water, dried in the shade, powdered mechanically, weighed, and stored in airtight jars. One litre of ethanol (95% v/v) was added to 250 g of powdered Azadirachta indica for 3 to 4 days. The mixture was stirred with a sterile glass rod after 12 h and was filtered with Whatman filter paper No. 1 three times. In a rotary evaporator, the solvent was removed under reduced pressure at a temperature of less than 50 °C, leaving a dark green residue stored in the airtight glass jars at 4 °C. The extract's weight was recorded and the percentage yield was 10.6% (Bhat et al. 2007). Moreover, a qualitative phytochemical analysis of the Azadirachta indica leaves was carried out.

Preparation of gel formulation

Distilled water was added to the Carbopol 940 and left overnight. To this mixture, triethanolamine was added vigorously. In water bath with a temperature not exceeding 50 °C, the Azadirachta indica extracts in a concentration of 1, 2, and 3 g were added to prepare three formulations, Gel-I (1% w/w), Gel-II (2% w/w) and Gel-III (3% w/w), respectively. Separately dissolved methyl and propylparaben in water were also added to this gel. Propylene glycol and polyethylene glycol were mixed in a separate beaker and added to this gel. The remaining quantity of purified
water was added, and the pH was dropwise adjusted with triethanolamine. The final weight was adjusted with water quantum statis (q.s.) to 100 g (Table 1).

**Evaluation of gels**

*Physical appearance.* The gel formulations were evaluated for their physical parameters like colour, odour, consistency, transparency, and homogeneity.

*Spreadability.* A glass slide with standard dimensions was used, where 0.5 g of the gel was placed in a circle 1 cm in diameter on the glass slide, over which another glass slide was placed. A weight of 125 g was set for 5 min so that the gel was sandwiched between the two slides to form a thin layer. Then, the weight was removed and the extra gel was removed. Then the slides were adjusted so that the upper slide was fixed with a weight of about 20 g. The time was noted for the slides to separate from each other (Prasad 2002). The spreadability was recorded using the following formula:

\[ S = \frac{M}{T} \quad (1) \]

where:

- \( S \) – spreadability in grams/seconds;
- \( M \) – mass in grams;
- \( T \) – time in seconds.

*Viscosity.* A Brookfield DV-E viscometer (RVDVE) was used to determine the viscosity of the gels. Spindle No. 07 was inserted in each formulation and was sheared at 3.3, 9.9, and 16.5 g at 24 ± 1 °C.

*Extrudability.* Twenty grams of each formulation was accurately weighed and packed in collapsible tubes, firmly pressed on one side which were then clamped. The cap was removed to allow the gel to extrude out, the gel was collected and weighed, the gel percentage was calculated (Aiyalu et al. 2016).

*Stability study of gels.* The gels were packed in collapsible airtight tubes and were stored at 8 °C (refrigerator temperature), 37 °C and 40 °C at 75 ± 1% relative humidity (RH) (an accelerated stability study) for three months. The samples were periodically taken out after one month and analysed for the physical tests for the colour, consistency, odour, spreadability, extrudability, viscosity, and pH (Bhowmik et al. 2009).

*Skin irritation test.* Six albino rabbits with about 1.8 kg in weight were used for this test. The animals were maintained at standard conditions (12/12 h light/dark cycle; 23 ± 1 °C, 35–60% RH). They were provided with water ad libitum. The irritation test was performed on the shaved back of the rabbit’s skin. Fifty milligrams of each gel was applied over one square centimetre area of the intact rabbit skin and observed for any oedema and erythema (Nawanopparatsakul et al. 2005).

**Antibacterial activity**

The antibacterial tests were performed in the Pathology and Microbiology Laboratory at The Children’s Hospital and Institute of Child Health, Multan. Standard strains of microorganisms (*Staphylococcus aureus* CECT 435, *Escherichia coli* CECT 943, *Pseudomonas aeruginosa* CECT 724) were taken from the Pathology Laboratory of the Food and Nutrition Department, Bahauddin Zakariya University (BZU), Multan. Each formulation was assessed for its antimicrobial effects against the microorganisms on a nutrient agar using a suitable diffusion method. About 0.2 ml of the bacterial test strain was inoculated over a nutrient agar plate with a sterile cotton swab and was al-

<table>
<thead>
<tr>
<th>Ingredients percentage</th>
<th>Gel-I</th>
<th>Gel-II</th>
<th>Gel-III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azadirachta indica extract (w/w)</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Carbopol 940</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Polyethylene glycol</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Propylene glycol 200</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Methyl paraben</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Propyl paraben</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Triethanolamine/sodium hydroxide 10%</td>
<td>q.s.n.</td>
<td>q.s.n.</td>
<td>q.s.n.</td>
</tr>
<tr>
<td>Purified water</td>
<td>q.s.p.</td>
<td>q.s.p.</td>
<td>q.s.p.</td>
</tr>
</tbody>
</table>

q.s.n. = quantity sufficient to neutralize gel base, q.s.p = quantity sufficient to prepare 100 grams of gel
allowed to dry. With the help of a cork borer, 6 mm diameter wells were created. Half a millilitre of the *Azadiracta indica* extract was introduced into the wells. The plates were placed at room temperature for about one hour. Then the plates were placed in an incubator at 37 °C for 24 hours. Then, the zone of inhibition was checked and recorded.

**Animals**

Albino rabbits with a weight range of 1.5–1.8 kg were placed in polypropylene cages (2 rabbits per cage). The temperature was maintained at 24 ± 1 °C, 40–60% relative humidity (RH), 12/12 h dark/light cycle. Water and feed were given *ad libitum*. The study was conducted based on an approval from the Institutional Animal Ethics Committee, BZU, Multan letter No. 014/PHP/EC-17 dated 07/10/2017.

**Design of study**

The animals were divided into five groups, having six animals each. Group I was considered as a control that received no treatment. Group II, III, IV, and V received a standard drug (Nitrofurazone), Gel-I containing 1% w/w of the *Azadiracta indica* extract, Gel-II containing 2% w/w of the *Azadiracta indica* extract, and Gel-III containing 3% w/w of the *Azadiracta indica* extract. No other medicine was given to the animals during the entire study. Any infected animals were replaced with fresh ones.

**Wound healing activity of gel**

The rabbits were anaesthetised before the wound creation, then 0.2 ml lignocaine HCl 2% (Barrett Hodgson Pharmaceuticals, Karachi, Pakistan) at a dose of 4 mg/kg body weight was given to each animal. The dorsal fur was shaved with the help of electric clippers, and the wound area was marked on the back of the animals. A linear incision wound having a length of 2.5 cm was created with a surgical blade to the depth of the subcutaneous tissues (0.5 cm deep) in sterile conditions. The animals were kept in separate cages after the wound creation. The day on which the wound was created was considered as day-0 (zero). The percentage of the wound contraction was considered a reduction in the wound length and was recorded on day 4, 8, 12, 16, and 20. A sterile scale was used to measure the size of the wound. The number of days required for the falling of the scab showed the period of epithelisation. The gels were applied on a wound once a day for 20 days. The epithelialisation time was noted by counting the number of days for falling of scar, leaving no wound behind. The wound contraction time was monitored by recording the wound length. The percentage of the wound contraction was measured from this area using the following equation (Gurung and Skalko-Basnet 2009):

\[
\text{Wound contraction} (%)= 100 \times \frac{(\text{first day wound size} - \text{wound size on specific day})}{\text{first day wound size}} \tag{2}
\]

**Histopathological study**

After anesthetising the animals, skin samples were taken for the histopathological study on day 0, 5, and 15. A 10% buffered formalin was applied to fixate the tissues. Using different grades of alcohol, the samples were fixed in paraffin wax. Haematoxylin and eosin (H&E) were used for the staining. The epithelialisation, keratinisation, collagen formation, fibrosis, and neovascularisation were examined under a microscope (Labomed America, Fremont, USA) (Gurung and Skalko-Basnet 2009).

**Statistical analysis**

In this analysis, the data were analysed using a one-way ANOVA (analysis of variance). The difference between the control and the treatments was considered significant if \( P < 0.05 \).

**RESULTS AND DISCUSSION**

This study evaluated the wound healing potential of herbal gels. Three different concentrations of an *Azadiracta indica* extract were used to prepare gel formulations with Carbopol 940. The formulations were evaluated for the physical parameters like the pH, viscosity, spreadability, and extrudability. Stability studies were carried out to ensure that the gels were stable at different temperatures maintaining the integrity and physicochemical features.
A pharmacological evaluation, like a skin irritation test, revealed that the herbal gels were safe to apply on the skin. The antibacterial activity of these gels against the different bacteria was also tested and confirmed. A wound healing study was carried out to show that the herbal gels can heal the wound without any infection.

**Phytochemical analysis of *Azadirachta indica***

Many phytochemicals were found in the ethanolic extract. Different tests were performed according to the standard methods to check for the presence of phytoconstituents such as alkaloids, flavonoids, tannins, reducing sugars, saponins, and triterpene glycosides in the ethanolic extract of the neem. The observations were recorded in Table 2.

**Evaluation of gel**

All the formulations were green. The spreadability indicates the extent to which the gel readily spreads on application to the skin or the affected part (the wound). The bioavailability efficiency of a gel formulation also depends on its spreading value. The extrudability reflects the gel's capacity to become uniformly ejected and to reach the desired quantity when the tube is squeezed. The results of the viscosity are also shown in Table 3.

**Stability studies.** The formulations were not affected by the temperature and maintained their integrity and physical features. The pH was in a range of 6 to 7. The drug content was also in the range of 90% to 105% for all the gels (Table 4).

### Table 2. Phytochemical constituents of ethanolic extract of *Azadirachta indica* leaves

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Constituent</th>
<th>Test name</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>glycoside</td>
<td>Legal's test</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>alkaloids</td>
<td>Mayer's reagent test</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>triterpenoids and steroids</td>
<td>Libermann test</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>flavonoids</td>
<td>Alkaline reagent test</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>reducing sugars</td>
<td>Fehling's test</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>carbohydrates</td>
<td>Molish's test</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>tannins</td>
<td>Ferric chloride test</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>saponins</td>
<td>Froth test</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>proteins and amino acids</td>
<td>Ninhydrin test</td>
<td>–</td>
</tr>
</tbody>
</table>

+ = means detected; – = means not detected

### Table 3. Physical evaluation of gels

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Colour</th>
<th>Appearance</th>
<th>pH</th>
<th>Spreadability (g.cm/sec)</th>
<th>Viscosity (cps)</th>
<th>Extrudability</th>
<th>Homogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gel-I</td>
<td>green</td>
<td>greasy transparent</td>
<td>6.78</td>
<td>36</td>
<td>55 400</td>
<td>excellent</td>
<td>homogenous</td>
</tr>
<tr>
<td>Gel-II</td>
<td>dark green</td>
<td>greasy translucent</td>
<td>6.69</td>
<td>33</td>
<td>60 200</td>
<td>good</td>
<td>homogenous</td>
</tr>
<tr>
<td>Gel-III</td>
<td>dark green</td>
<td>greasy translucent</td>
<td>6.81</td>
<td>31</td>
<td>64 300</td>
<td>good</td>
<td>homogenous</td>
</tr>
</tbody>
</table>

### Table 4. Stability study parameters of gels at different temperatures

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Gel-I</th>
<th>Gel-II</th>
<th>Gel-III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>T&lt;sub&gt;2&lt;/sub&gt;</td>
<td>T&lt;sub&gt;3&lt;/sub&gt;</td>
</tr>
<tr>
<td>Appearance</td>
<td>green transparent</td>
<td>dark green translucent</td>
<td>dark green translucent</td>
</tr>
<tr>
<td>Nature</td>
<td>hm.</td>
<td>hm.</td>
<td>hm.</td>
</tr>
<tr>
<td>Viscosity (cps)</td>
<td>61 340</td>
<td>55 400</td>
<td>55 250</td>
</tr>
<tr>
<td>Extrudability</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Spreadability (g.cm/sec)</td>
<td>34.68</td>
<td>34.68</td>
<td>34.68</td>
</tr>
<tr>
<td>Phase separation</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
</tbody>
</table>

Hm. = homogeneous; T<sub>1</sub> = refrigerator; T<sub>2</sub> = room temperature; T<sub>3</sub> = controlled (40 ± 0.5 °C)
Skin irritation test. All the gel formulations were found to be safe while being applied on the skin and there was no irritation or sensitivity to the skin.

Antibacterial activity

The antibacterial activity showed (Table 5) that the zone of inhibition increased with an increase in the concentration of the herbal extract. It indicates that the Azadirachta indica leaf extract possesses an antibacterial activity, helps maintain a sterile wound area, and promotes the wound healing process. Gel-III was found to be more effective in the wound healing when compared to other herbal gels. These gels showed better activity against Escherichia coli and Pseudomonas aeruginosa when compared to Staphylococcus aureus.

Koona and Budida (2011) reported the antibacterial activity of a methanolic leaf extract of Azadirachta indica against E. coli. Additionally, an Aloe vera extract was used to study its antibacterial effect against P. aeruginosa, S. aureus, and E. coli (Arunkumar and Muthuselvam 2009).

The antimicrobial activity against various microorganisms like S. aureus E. coli and Bacillus subtilis bacteria was evaluated. It was reported that the Azadirachta indica extract was effective against all microorganisms when compared to other plant extracts and the standard ofloxacin (Kumar et al. 2007). Priadarshini et al. (2013) studied the antibacterial activity of an extract (200, 150, 100, 50, and 25 mg/ml concentrations) obtained from leaves of herbs like Azadirachta indica and Moringa oleifera against microorganisms.

The results were compared with the standard drug, gentamycin. Both plants’ extracts showed activity against microorganisms like Escherichia coli, Klebsiella pneumonia, Proteus vulgaris, Bacillus subtilis, and Pseudomonas aeruginosa in ascending order (Priadarshini et al. 2013).

Wound healing activity of gel

Gel-III containing 3% w/w showed a better healing activity when compared to Gel-I and Gel-II. The rabbits’ skin treated with the 3% w/w Azadirachta indica extract epithelialised in 23 days compared to the control and standard drug where the epithelialisation occurred in 34 and 26 days, respectively (Figures 1, 2 and 3). The percentage reduction in the wound area was studied on the 20th day and was

Table 5. Antibacterial activity (zone of inhibition shown by gel formulations)

<table>
<thead>
<tr>
<th>Formulation</th>
<th>S. aureus (mm)</th>
<th>E. coli (mm)</th>
<th>P. aeruginosa (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>16.1 ± 0.9</td>
<td>16.2 ± 0.7</td>
<td>19.3 ± 0.5</td>
</tr>
<tr>
<td>Gel-I</td>
<td>11.3 ± 0.5</td>
<td>13.5 ± 0.4</td>
<td>12.4 ± 0.8</td>
</tr>
<tr>
<td>Gel-II</td>
<td>12.1 ± 0.3</td>
<td>15.4 ± 0.2</td>
<td>13.1 ± 0.6</td>
</tr>
<tr>
<td>Gel-III</td>
<td>14.3 ± 0.3</td>
<td>16.2 ± 0.6</td>
<td>15.6 ± 0.6</td>
</tr>
</tbody>
</table>

![Figure 1. Animals treated with Gel-I containing 1% w/w Azadirachta indica extract](image1)

![Figure 2. Animals treated with Gel-II containing 2% w/w Azadirachta indica extract](image2)
Figure 3. Animals treated with Gel-III containing 3% w/w Azadirachta indica extract

63.68, 90.53, 88.76, 92.32, and 96.41% for the control, the standard drug, Gel-I, Gel-II, and Gel-III treated animals, respectively, as shown in Table 6. The difference was statistically significant ($P < 0.05$) between control and Gel-III.

**Histopathological study**

The histopathological studies revealed the suitability of the gels in the wound healing (Table 7). The microscopic images of the skin samples stained by H&E are shown in Figures 4 and 5 for the 5th and 15th day of the post-wounding, respectively.

**Table 6. Effect of gel formulations on wound healing**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Wound contraction in percentage</th>
<th>Epithelization time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4th day</td>
<td>8th day</td>
</tr>
<tr>
<td>Control</td>
<td>3.86 ± 1.04</td>
<td>10.82 ± 1.55</td>
</tr>
<tr>
<td>Standard</td>
<td>6.21 ± 1.23</td>
<td>26.35 ± 1.54</td>
</tr>
<tr>
<td>Gel-I</td>
<td>5.01 ± 1.21</td>
<td>24.16 ± 1.91</td>
</tr>
<tr>
<td>Gel-II</td>
<td>5.88 ± 1.62</td>
<td>25.12 ± 1.71</td>
</tr>
<tr>
<td>Gel-III</td>
<td>6.12 ± 1.23</td>
<td>26.11 ± 1.43</td>
</tr>
</tbody>
</table>

All values are mean ± standard deviation of six animals in each group. All values are significant at $P < 0.05$ vs control

**Table 7. Histological scoring of wound on 15th day after surgery**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Inflammation</th>
<th>Proliferation</th>
<th>Remodeling</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>neutrophils</td>
<td>macrophages</td>
<td>fibrosis</td>
</tr>
<tr>
<td>Control</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Standard</td>
<td>+++</td>
<td>–</td>
<td>+++</td>
</tr>
<tr>
<td>Gel-I</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gel-II</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gel-III</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
</tbody>
</table>

– = none; + = minimal; ++ = mild; +++ = high

All values are mean ± standard deviation of six animals in each group. All values are significant at $P < 0.05$ vs control
of fibroblasts, hair-follicles, while the blood vessels were also prominent.

On the 15th day, samples were again taken and stained with H&E. The photomicrograph (in Figure 5A) of the control animal’s skin showed a low number of neutrophils where the neovascularisation was very slow, and the number of blood vessels was also low. The photomicrograph (in Figure 5B) of the animal’s skin treated with the standard gel showed a prominent epidermis and faster rate of epithelialisation, the number of neutrophils and fibroblasts were greater than the hair follicles seen. The photomicrograph (in Figure 5C) of the skin treated with Gel-I showed an abundant number of neutrophils and connective tissues were well in contact. The photomicrograph (in Figure 5D) of the skin treated with Gel-II showed a smaller number of macrophages and few fibroblasts. The photomicrograph (in Figure 5E) of the skin treated with Gel-III showed macrophages were well organised and abundant.

The different concentration gels showed promising wound-healing effects; the present results were also compared with previous studies. A paste prepared from the *Azadirachta indica* bark was applied on the excision and incision wounds in mice in an earlier study (Maan et al. 2017). The findings showed excellent activity in the wound contraction, histopathology, and breaking strength. The values of all the parameters revealed that the neem extract had an excellent activity in the wound healing (Maan et al. 2017). Alzohairy (2016) reviewed the therapeutic activity of *Azadirachta indica* and found its anti-inflammatory activity in rats after an oral dose. The leaf extract of neem showed a wound healing activity in Sprague-Dawley rats. The tensile strength of the wound tissue was also higher in the neem treated group when compared to the standard group. *Azadirachta indica* promoted the wound healing through a better neovascularisation and inflammatory response. In our
study, the histological results revealed a better neo-
vascularisation and epithelialisation. *Azadirachta indica* leaves contain various phytochemicals that
possess an intense activity against bacteria, vi-
ruses, and fungi (Chundran et al. 2015). Sodium
nimbidate and the nimbidin present in the neem
also have anti-inflammatory and wound healing
properties. The formation of collagen and hair
follicles occurs rapidly because of the nutrients
present in the neem leaves (Chundran et al. 2015).
In the present study, the collagen synthesis and hair
formation were likely increased with the increasing
gel concentration. Acemannan is a polysaccharide
of aloe vera that stimulates the proliferation, vas-
cular endothelial growth factor (VEGF), kerati-
nocyte growth factor-1 (KGF 1), and oral wound
healing in rats, which potentiate the wound heal-
ing (Jettanacheawchankit et al. 2009). The addi-
tion of aloe vera in a neem gel further enhances
the wound-healing effects. The *Azadirachta indica*
treated animals resulted in abundant angiogenesis
by the proliferation of the connective tissues and
fibroblastic deposition. The angiogenesis was en-
hanced in animals treated with *Azadirachta indica* (Barua et al. 2010). The histological analysis
of wounds treated with *Azadirachta indica* showed
the proliferation of fibroblasts, neovascularisation,
and collagen synthesis, which accelerated the
wound healing (Osunwoke Emeka et al. 2013).

According to the present study, the wound
contraction improves with the increasing con-
centration of the herbal extract. Out of the gel for-
formulations containing an *Azadirachta indica* ex-
tract in the concentration of 1, 2, and 3% w/w, a
formulation containing 3% w/w extract of *Azadirachta indica* showed better wound healing and anti-
microbial effects, concluding that the extract
of *Azadirachta indica* (3% w/w) was a better can-
didate for wound healing.

**Conflict of interest**

The authors declare no conflict of interest.
REFERENCES


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