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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.,

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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). Herbals 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, antimycotic, 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

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Table 1. Composition of gel formulations

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

q.s.n. = quantity sufficient to neutralize gel base, q.s.p = quantity sufficient to prepare 100 grams of gel

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:

$$S = M/T \quad (1)$$

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. the gel formulations were prepared in distilled water (Vador et al. 2012).

pH. The pH of the gels was detected with a digital pH meter (Transmark 126, Nanjing, P.R. China). An amount of 0.5 g of gel was dissolved in 50 ml of distilled water and stored for two hours. Each formulation's pH was measured in triplicate and the average values were taken (Derle et al. 2006).

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-

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lowed to dry. With the help of a cork borer, 6 mm diameter wells were created. Half a millilitre of the *Azadirachta 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 *Azadirachta indica* extract, Gel-II containing 2% w/w of the *Azadirachta indica* extract, and Gel-III containing 3% w/w of the *Azadirachta 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 anesthetised 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):

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

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 *Azadirachta 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.

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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.

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

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

+ = means detected; – = means not detected

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 3. Physical evaluation of gels

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

Table 4. Stability study parameters of gels at different temperatures

Parameter	Gel-I			Gel-II			Gel-III		
	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3
Appearance	green transparent	dark green translucent	dark green translucent	green transparent	dark green translucent	dark green translucent	green transparent	dark green translucent	dark green translucent
Nature	hm.	hm.	hm.	hm.	hm.	hm.	hm.	hm.	hm.
Viscosity (cps)	61 340	55 400	55 250	61 100	60 430	53 270	65 400	64 360	54 780
Extrudability	+	+++	+++	+	++	++	+	++	++
Spreadability (g.cm/sec)	34.68	34.68	34.68	34.68	34.68	34.68	34.68	34.68	34.68
pH	6.46	6.46	6.46	6.46	6.46	6.46	6.46	6.46	6.46
Phase separation	no	no	no	no	no	no	no	no	no

Hm. = homogeneous; T_1 = refrigerator; T_2 = room temperature; T_3 = controlled (40 ± 0.5 °C)

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

Formulation	*S. aureus* (mm)	*E. coli* (mm)	*P. aeruginosa* (mm)
Standard	16.1 ± 0.9	16.2 ± 0.7	19.3 ± 0.5
Gel-I	11.3 ± 0.5	13.5 ± 0.4	12.4 ± 0.8
Gel-II	12.1 ± 0.3	15.4 ± 0.2	13.1 ± 0.6
Gel-III	14.3 ± 0.3	16.2 ± 0.6	15.6 ± 0.6

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.*

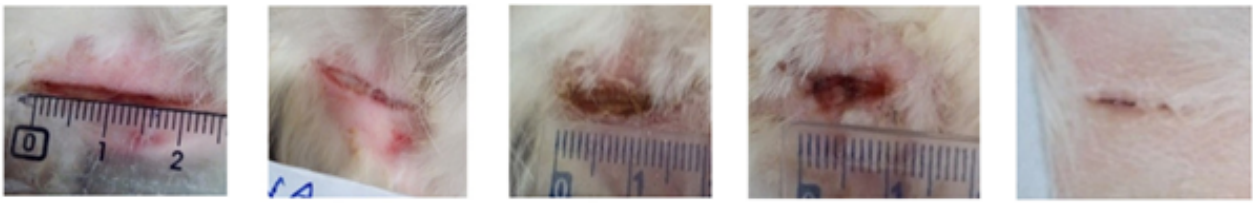
Koona and Budida (2011) reported the antibacterial activity of a methanolic leaf extract of *Azadirachta indica* against *E. coli*. Additionally, an *Aloe vera* extract was used to study its antibacterial effect against *P. aeruginosa*, *S. aureus*, and *E. coli* (Arunkumar and Muthuselvam 2009).

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).

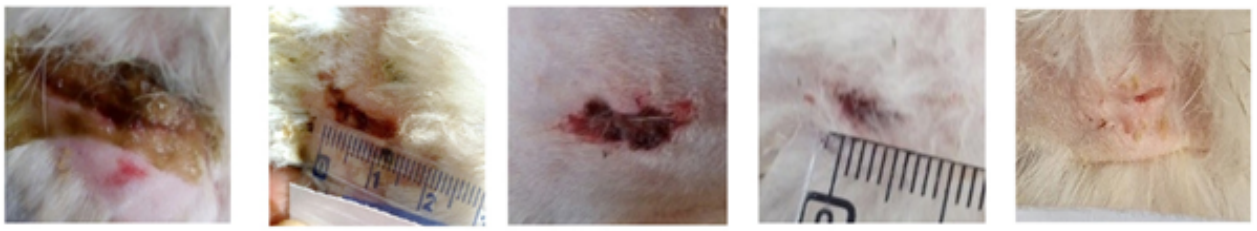
Wound healing activity of gel

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



4th day 8th day 12th day 16th day 20th day

Figure 1. Animals treated with Gel-I containing 1% w/w *Azadirachta indica* extract



4th day 8th day 12th day 16th day 20th day

Figure 2. Animals treated with Gel-II containing 2% w/w *Azadirachta indica* extract

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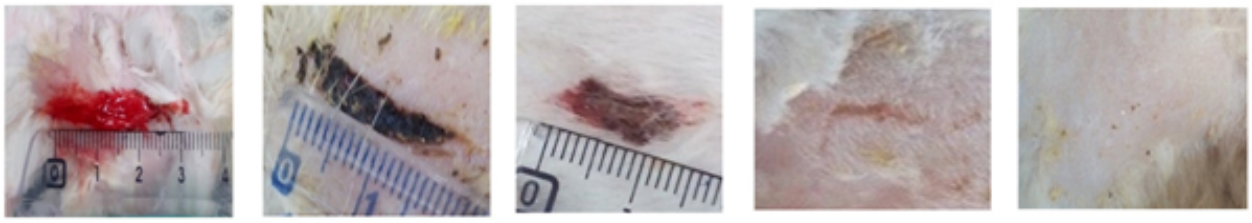

4th day 8th day 12th day 16th day 20th day

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.

Figure 4 shows the animal skin that received no treatment (control) with hair-follicles and macrophages around the epidermis. The photomicrograph (in Figure 4B) of a section taken from an animal treated with the standard gel showed well-established connective tissues, and re-epithelialisation was seen in some areas, neutrophils gathered around the blood vessels, which further resulted in angiogenesis. The photomicrograph (Figure 4C) of the skin that was treated with Gel-I showed fewer neutrophils and blood vessels. The skin section (in Figure 4D) treated with Gel-II showed a lower number of macrophages. The photomicrograph (in Figure 4E) of the skin sample treated with Gel-III showed an abundant number

Table 6. Effect of gel formulations on wound healing

Treatment	Wound contraction in percentage					Epithelization time (days)
	4th day	8th day	12th day	16th day	20th day	
Control	3.86 ± 1.04	10.82 ± 1.55	21.13 ± 1.09	50.08 ± 2.11	63.68 ± 2.17	34.00 ± 1.88
Standard	6.21 ± 1.23	26.35 ± 1.54	49.33 ± 1.89	72.76 ± 1.65	90.53 ± 1.23	26.00 ± 1.85
Gel-I	5.01 ± 1.21	24.16 ± 1.91	43.21 ± 2.36	69.82 ± 1.55	88.76 ± 2.15	27.00 ± 1.36
Gel-II	5.88 ± 1.62	25.12 ± 1.71	47.32 ± 2.01	73.99 ± 1.42	92.32 ± 1.82	25.00 ± 2.03
Gel-III	6.12 ± 1.23	26.11 ± 1.43	55.23 ± 1.89	78.97 ± 2.15	96.41 ± 2.10	23.00 ± 2.01

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

Treatment	Inflammation			Proliferation		Remodeling
	neutrophils	macrophages	fibrosis	re-epithelialization	neovascularization/ angiogenesis	collagen deposition
Control	++	++	++	–	–	++
Standard	+++	–	+++	+++	+++	+++
Gel-I	+++	+	+	+	+	++
Gel-II	+	+	+	+	+	+
Gel-III	+	+++	+++	++	++	++

– = 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

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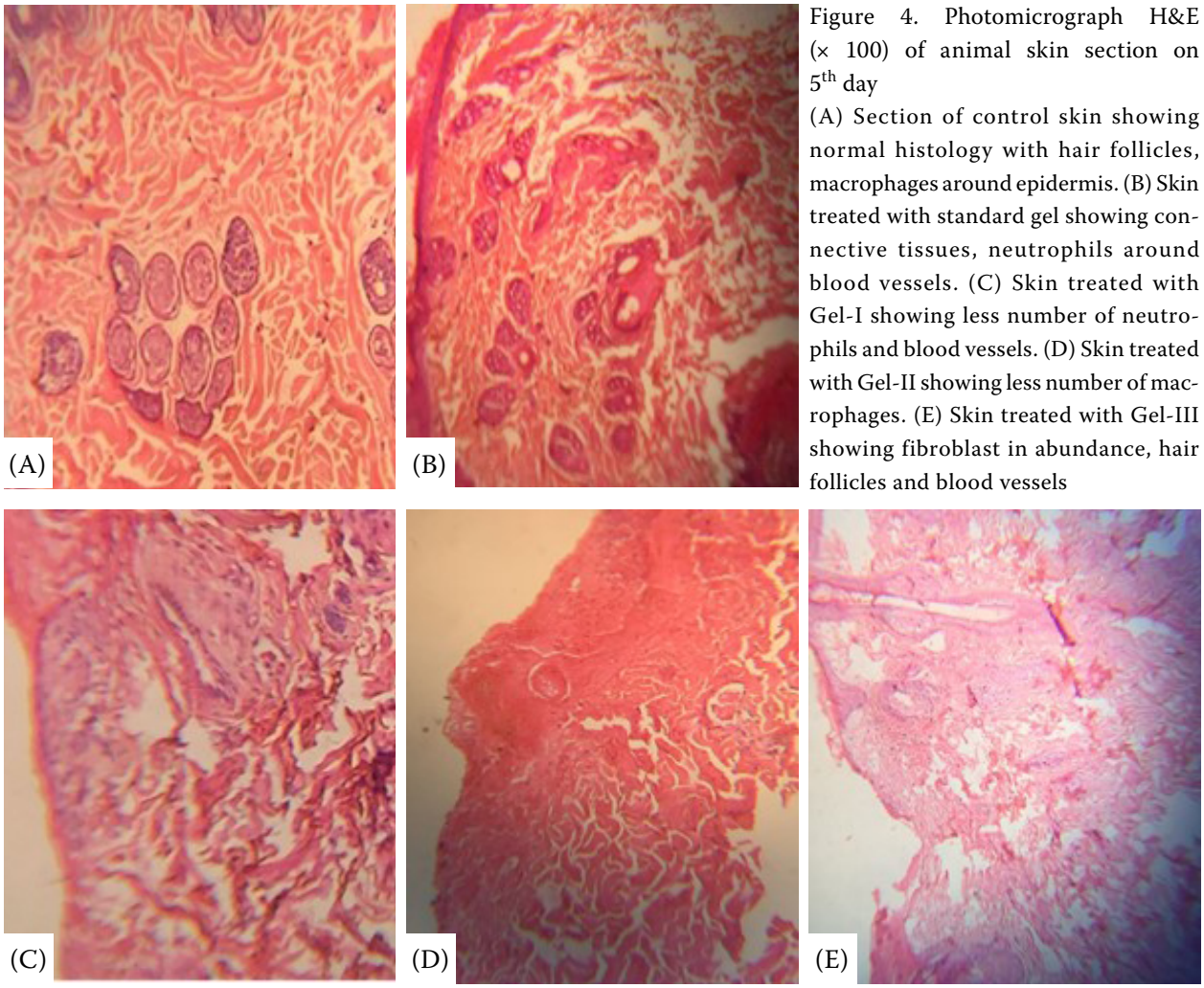

Figure 4. Photomicrograph H&E (× 100) of animal skin section on 5th day
(A) Section of control skin showing normal histology with hair follicles, macrophages around epidermis. (B) Skin treated with standard gel showing connective tissues, neutrophils around blood vessels. (C) Skin treated with Gel-I showing less number of neutrophils and blood vessels. (D) Skin treated with Gel-II showing less number of macrophages. (E) Skin treated with Gel-III showing fibroblast in abundance, hair follicles and blood vessels

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

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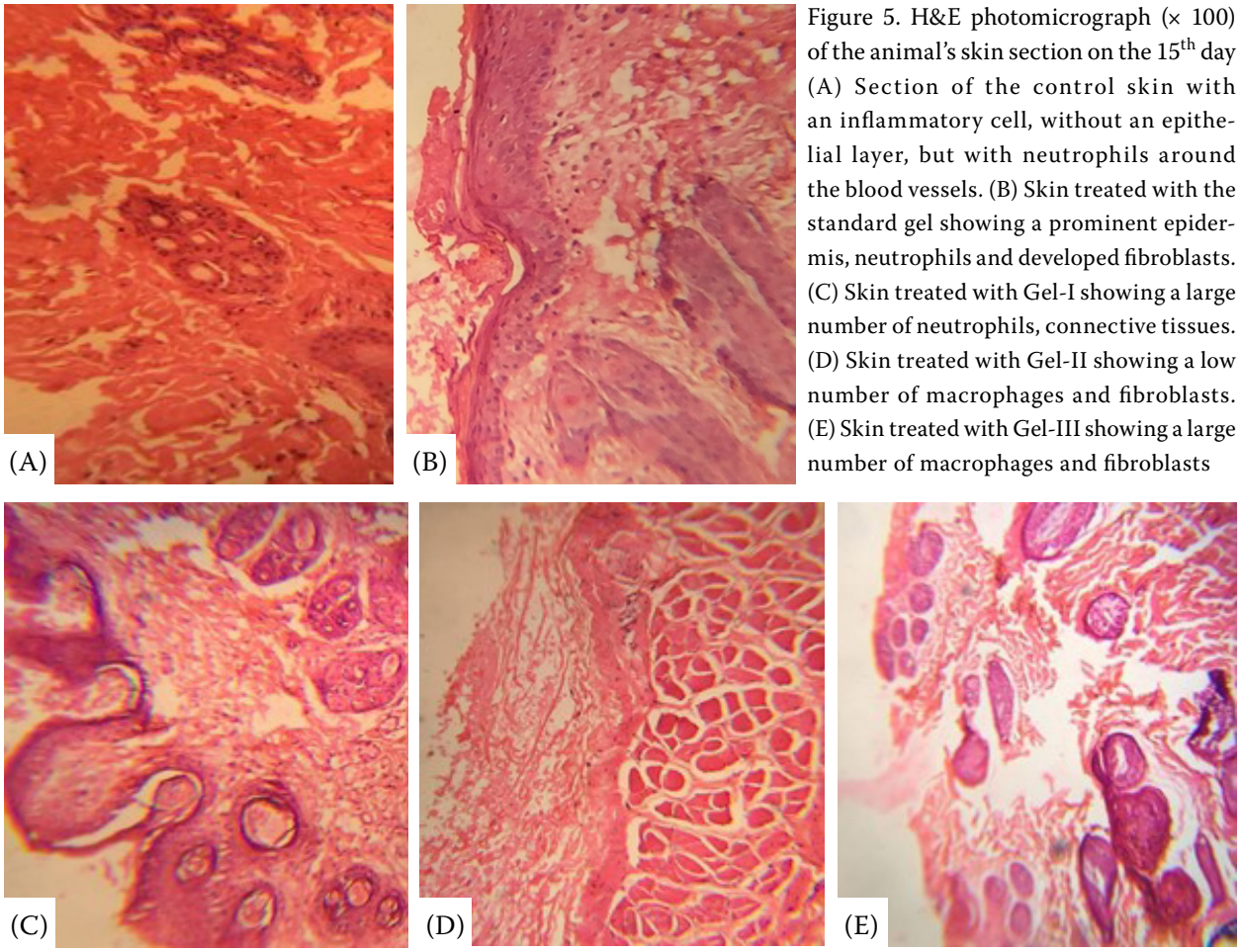

Figure 5. H&E photomicrograph (× 100) of the animal's skin section on the 15th day (A) Section of the control skin with an inflammatory cell, without an epithelial layer, but with neutrophils around the blood vessels. (B) Skin treated with the standard gel showing a prominent epidermis, neutrophils and developed fibroblasts. (C) Skin treated with Gel-I showing a large number of neutrophils, connective tissues. (D) Skin treated with Gel-II showing a low number of macrophages and fibroblasts. (E) Skin treated with Gel-III showing a large number of macrophages and fibroblasts

study, the histological results revealed a better neovascularisation and epithelialisation. *Azadirachta indica* leaves contain various phytochemicals that possess an intense activity against bacteria, viruses, 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, vascular endothelial growth factor (VEGF), keratinocyte growth factor-1 (KGF 1), and oral wound healing in rats, which potentiate the wound healing (Jettanacheawchankit et al. 2009). The addition 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 enhanced 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 concentration of the herbal extract. Out of the gel formulations containing an *Azadirachta indica* extract 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 antimicrobial effects, concluding that the extract of *Azadirachta indica* (3% w/w) was a better candidate for wound healing.

Conflict of interest

The authors declare no conflict of interest.

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REFERENCES

Aiyalu R, Govindarjan A, Ramasamy A. Formulation and evaluation of topical herbal gel for the treatment of arthritis in animal model. Braz J Pharm Sci. 2016 Jul-Sep; 52(3):493-507.

Alzohairy MA. Therapeutics role of Azadirachta indica (neem) and their active constituents in diseases prevention and treatment. Evid Based Complement Alternat Med. 2016;2016: [11].

Arunkumar S, Muthuselvam M. Analysis of phytochemical constituents and antimicrobial activities of Aloe vera L. against clinical pathogens. World J Agr Sci. 2009;5(5): 572-6.

Ayello EA. What does the wound say? Why determining etiology is essential for appropriate wound care. Adv Skin Wound Care. 2005 Mar;18(2):98-109.

Barua C, Talukdar A, Barua A, Chakraborty A, Sarma R, Bora R. Evaluation of the wound healing activity of methanolic extract of Azadirachta Indica (neem) and Tinospora cordifolia (guduchi) in rats. Pharmacologyonline. 2010;1:70-7.

Bhat RS, Shankrappa J, Shivakumar H. Formulation and evaluation of polyherbal wound treatments. Asian J Pharm Sci. 2007;2(1):11-7.

Bhowmik BB, Nayak BS, Chatterjee A. Formulation development and characterization of metronidazole microencapsulated bioadhesive vaginal gel. Int J Pharm Pharm Sci. 2009 Jul-Sep;1(1):240-57.

Biswas K, Chattopadhyay I, Banerjee RK, Bandyopadhyay U. Biological activities and medicinal properties of neem (Azadirachta indica). Curr Sci. 2002 Jun;82(11):1336-45.

Chen KX. Academician kai-xian chen talks about the development of traditional chinese medicine and global medicine. World J Tradit Chin Med. 2020;6(1): [11].

Chundran NK, Husen IR, Rubianti I. Effect of neem leaves extract (Azadirachta Indica) on wound healing. Althea Med J. 2015 Jun;2(2):199-203.

Derle D, Sagar B, Kotwal R, Ingole R, Chavhan S. A comparative in vitro evaluation of transdermal permeation of valdecoxib and its complex with HP-β- Cyclodextrin from microemulsion based gel. Indian Drugs. 2006;43(8):625.

Gao L, Jia CH, Wang W. Recent advances in the study of ancient books on traditional Chinese medicine. World J Tradit Chin Med. 2020;6(1):61-6.

Joshi B, Sah GP, Basnet BB, Bhatt MR, Sharma D, Subedi K, Pandey J, Malla R. Phytochemical extraction and antimicrobial properties of different medicinal plants: Ocimum sanctum (tulsi), Eugenia caryophyllata (clove), Achyranthes bidentata (datiwan) and Azadirachta indica (neem). J Microbiol Antimicrob. 2011;3(1): [7].

Gupta AK, Ahirwar NK, Shinde N, Choudhary M, Rajput YS, Singh A. Phytochemical screening and antimicrobial assessment of leaves of Adhatoda vasica, Azadirachta indica and Datura stramonium. UK J Pharm Biosci. 2013; 1(1):42-7.

Gurung S, Skalko-Basnet N. Wound healing properties of Carica papaya latex: In vivo evaluation in mice burn model. J Ethnopharmacol. 2009 Jan 21;121(2):338-41.

Jettanacheawchankit S, Sasithanasate S, Sangvanich P, Banlunara W, Thunyakitpisal P. Acemannan stimulates gingival fibroblast proliferation; expressions of keratinocyte growth factor-1, vascular endothelial growth factor, and type I collagen; and wound healing. J Pharmacol Sci. 2009 Apr;109(4):525-31.

Koona S, Budida S. Antibacterial potential of the extracts of the leaves of Azadirachta indica Linn. Not Sci Biol. 2011; 3(1):65-9.

Krishnan P. The scientific study of herbal wound healing therapies: Current state of play. Curr Anaesth Crit Care. 2006;17(1-2):21-7.

Kumar B, Vijayakumar M, Govindarajan R, Pushpangadan P. Ethnopharmacological approaches to wound healing – Exploring medicinal plants of India. J Ethnopharmacol. 2007 Nov 1;114(2):103-13.

Maan P, Yadav KS, Yadav NP. Wound healing activity of Azadirachta indica A. juss stem bark in mice. Pharmacogn Mag. 2017 Jul;13(Suppl 2):316-20.

Maithani A, Parcha V, Pant G, Dhulia I, Kumar D. Azadirachta indica (neem) leaf: A review. J Pharm Res. 2011;4(6): 1824-7.

Nawanopparatsakul S, Euasathien J, Eamtawecharum C, Benjasirimingokol P, Soiputtan S, Toprasri P, Phaechamud T. Skin irritation test of curcuminoids facial mask containing chitosan as a binder. Silpakorn University J. 2005;5(1-2):140-7.

Osunwoke Emeka A, Olotu Emamoke J, Allison Theodore A, Onyekwere Julius C. The wound healing effects of aqueous leave extracts of azadirachta indica on wistar rats. J Nat Sci Res. 2013;3(6):181-6.

Parrotta JA, Chaturvedi A. Azadirachta indica A. Juss. Neem, margosa. Meliaceae. Mahogany family. New Orleans: USDA Forest Service, International Institute of Tropical Forestry; 1994. 8 p.

Prasad V. Evaluation of indigenous formulations for wound healing activity [thesis]. Nagpur University, Nagpur, India; 2002. p. 7-8.

Priadarshini A, Pankaj PP, Varma M, Kumar K. Evaluation of the antibacterial potential of Moringa oleifera and Azadirachta indica against some pathogenic microbes: A comparative study. Int J Drug Dev & Res. 2013;5(1): 214-8.

https://doi.org/10.17221/46/2020-VETMED

Puri HS. Neem: The divine tree; Azadirachta indica. Amsterdam, The Netherlands: Harwood Academic Publishers; 1999. 183 p.

Sarimah M, Mizaton H. The wound healing activity of Mikania micrantha ehanolic leaf extract. J Fundam Appl Sci. 2018;10(6S):425-37.

Saleem S, Muhammad G, Hussain MA, Bukhari SNA. A comprehensive review of phytochemical profile, bioactives for pharmaceuticals, and pharmacological attributes of Azadirachta indica. Phytother Res. 2018 Jul;32(7): 1241-72.

Tong HY, Zhang SQ, Murtaza G, Zhao HH, Huang XJ, Hu-Rilebagen, Wu-Lanqiqige, Bao WY, Wu-Jisiguleng, Wu-Yunsiriguleng, Chen LY. The present scenario, challenges, and future anticipation of traditional Mongolian medicine in China. World J Tradit Chin Med. 2019;5(4):187-92.

Vador N, Vador B, Hole R. Simple spectrophotometric methods for standardizing ayurvedic formulation. Indian J Pharm Sci. 2012 Mar;74(2):161-3.

Yang SJ, Wang ZY, Zhao HH, Ren XQ. Modern research of tibetan medicine. World J Tradit Chin Med. 2019;5(2): 131-8.

Zhao K, Shi N, Sa Z, Wang HX, Lu CH, Xu XY. Text mining and analysis of treatise on febrile diseases based on natural language processing. World J Tradit Chin Med. 2020;6(1):67-73.

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